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A novel stability-indicating method for known and unknown impurities profiling for diltiazem hydrochloride pharmaceutical dosage form (tablets)

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Abstract

Background: A novel gradient, high-sensitive and specific stability-indicating reverse-phase HPLC method was developed and validated for quantitative purpose of known, unknown and degradant impurities profiling for diltiazem hydrochloride tablets. The impurities were separated on the Zorbax RX C8 column (150 mm × 4.6 mm, 5 µm) with mobile phase-A consisting of a mixture of 0.05 M sodium dihydrogen phosphate monohydrate buffer pH 3.0 and methanol in the ratio 800:200v/v and mobile phase-B consisting of acetonitrile with a flow rate of 1.0 mL min^{-1}. The column compartment was maintained at 35 °C, and the detection wavelength was 240 nm. Diltiazem hydrochloride, its known impurities and unknown impurities have been well resolved from each other.

Results: The linearity of the method has been demonstrated across the concentration range of 0.18 to 5.65 µg mL^{-1} for EP impurity-F with correlation coefficient R^2 greater than 0.99. Recovery of method was proved from LOQ to 150% for known and unknown impurities with respect to test concentration and found in between 80 and 120%. Forced degradation study and specificity experiment results with mass balance proved the stability-indicating nature of the method and separated all known, unknown impurities and degradants from each other as well as from main drug component (diltiazem hydrochloride). The mass balance for stress study was found in between 95 and 105%.

Conclusion: Newly developed analytical method was validated as per ICH Q2 (R1) guidelines "Validation of analytical procedure" and found linear, accurate, specific, robust and precise in the established working range.

Keywords: Diltiazem hydrochloride, Method development, Method validation, Impurities profiling, Stability indicating

Background

Diltiazem hydrochloride is chemically known as 3-(acetyloxy)-5-[2-(dimethylamino) ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-1, 5-benzothiazepin-4(5H)-one monohydrochloride (Fig. 1). Diltiazem hydrochloride is a calcium channel-blocking agent, nondihydropyridine derivative [1].

Its medication was used to treat high blood pressure, angina and certain heart arrhythmias [2]. It may also be used in hyperthyroidism if beta blockers cannot be used [3]. The drug product is considered safe when the levels of impurities (known and unknown) are below the maximum permeability limits during the shelf-life study according to ICH guidelines [4, 5]. The requirement of health agencies is that the impurities profiling analysis of drug substance and drug product during their shelf-life study was carried out using suitable validated analytical

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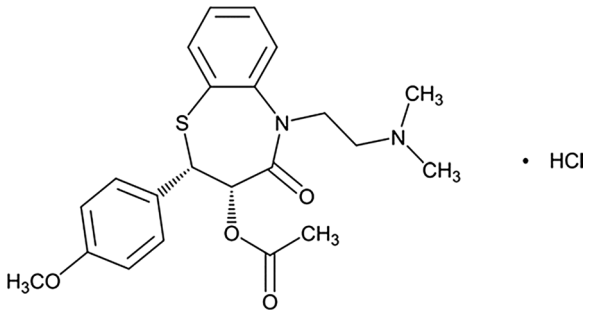

Fig. 1 Chemical structure of diltiazem hydrochloride

method [6–9]. The forced degradation study of drug product and drug substance is an important part in the development of analytical methods to demonstrate the stability-indicating power of the method. Forced degradation study of drug product and drug substance produces potential degradants impurities [10, 11]. The drug product and drug substance are subjected to stress study such as oxidation, acid hydrolysis, alkali hydrolysis, photolytic degradation, thermal degradation and humidity degradation.

The force degradation study will help identify unknown potential degradants and provide information necessary for the stability of drug substances and pharmaceutical products. Therefore, the results of the diltiazem hydrochloride degradation study, which will provide information on the intrinsic stability of the drug product, are reported here.

The literature survey revealed that the several RP-HPLC methods were reported for the determination of diltiazem hydrochloride alone [12–25] and in combination with other drugs or components [26–30].

Diltiazem hydrochloride drug substance (API) is cited in the British Pharmacopoeia [31] and European Pharmacopoeia [32] mentioned to monitor the following known impurities, i.e., impurities-A, B, C, D, E and F. US Pharmacopoeia is not discussed these impurities for diltiazem hydrochloride drug substance (API) [33]. For diltiazem hydrochloride pharmaceutical dosage form (tablets), no impurity profile method is available in British Pharmacopoeia and European Pharmacopoeia.

In US Pharmacopoeia for diltiazem hydrochloride pharmaceutical dosage form (tablets) monograph [34], impurities-A, B, C and D are mentioned, controlling these impurities under any unknown impurity, i.e., it indicates that these are process impurities and will not increase during stability. US Pharmacopoeia is not impurity-E and impurity-F profiling in pharmaceutical dosage form (tablets), while in European Pharmacopoeia for diltiazem hydrochloride drug substance (API) impurity-F is mentioned as degraded specified impurity and other impurities-A, B, C, D and E mentioned as unspecified impurity (controlled under any unknown impurity).

However, the comprehensive literature review found that very few methods were reported for the determination of related substances [35–37] and synthetic reagent [38] for diltiazem hydrochloride. The methods that have been reported for related substances by HPLC and HPLC–MS method [35] discussed characterization of impurities-A, B, E and F. An HPLC method for assay of diltiazem hydrochloride and its related substances (RS) in bulk drug and finished tablets was reported [36] without any comment on stability-indicating nature of the method and impurity profiling. The method was reported for related substances [37] having analysis run time about 90 min, which is time-consuming in routine testing of samples, and another concern is system suitability testing of method involving preparation of resolution solution and standard solution in which each time impurities consumption involves which is not economically feasible. The method did not discuss European Pharmacopoeia impurity-C profiling and degradation kinetics.

Therefore, it is essential to develop a reverse-phase liquid chromatographic procedure for the impurities profiling and degradation kinetics of known European Pharmacopoeia impurities-A, B, C, E and F for diltiazem hydrochloride pharmaceutical dosage form (tablets) that will serve a reliable, accurate, sensitive, rugged, robust, stability indicating and economically feasible method.

By considering all the above information, the objective of method development and validation is to prove the specificity for impurities-A, B, C, E and F and validate the analytical method for EP impurity-F which is a specified degradation product as per European Pharmacopoeia for diltiazem hydrochloride pharmaceutical dosage form (tablets).

The chemical names of diltiazem hydrochloride and its impurities are shown in Table 1. The chemical structures of diltiazem hydrochloride and its impurities are shown in Figs. 1, 2, 3, 4, 5 and 6.

Methods

Reagents and materials

Marketed samples of diltiazem hydrochloride tablets were used for analytical method development and analytical method validation. The related substances (impurities-A, B, C, E and F) of diltiazem hydrochloride were procured from Olympus Chemical and Fertilizer, Mumbai (India). Sodium dihydrogen phosphate monohydrate, methanol and acetonitrile were obtained from Spectrochem Limited, and HPLC-grade water was obtained from Milli-Q purification system. 0.45-µm PVDF filter was used of Merck, India make.

Table 1 Chemical name of Diltiazem and its impurities

Compound name	Chemical name	Molecular weight
Diltiazem hydrochloride	3-(Acetyloxy)-5-[2-(dimethylamino) ethyl]-2, 3-dihydro-2-(4-methoxyphenyl)-1, 5-benzothiazepin -4(5H)-one monohydrochloride	450.98
Diltiazem EP impurity-A	(2R,3S)-5-[2-(dimethylamino) ethyl]-2-(4-methoxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepine-3-yl acetate	414.52
Diltiazem EP impurity-B	(2S,3S)-2-(4-methoxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1, 5-benzothiazepine-3-yl acetate	343.40
Diltiazem EP impurity-C	(2S,3S)-5-[2-(dimethylamino)ethyl]-2-(4-hydroxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl acetate	400.49
Diltiazem EP impurity-E	(2S,3S)-3-hydroxy-2-(4-methoxyphenyl)-2,3-dihydro-1,5- benzothiazepin-4(5H)-one	301.36
Diltiazem EP impurity-F	(2S,3S)-5-[2-(dimethylamino)ethyl]-3-hydroxy-2-(4-hydroxyphenyl)-2,3-dihydro-1,5-benzothiazepin-4(5H)-one	372.5

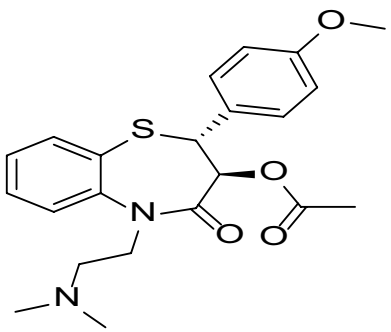

Fig. 2 Chemical structure of diltiazem EP impurity-A

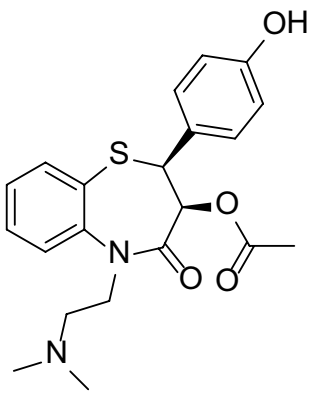

Fig. 4 Chemical structure of diltiazem EP impurity-C

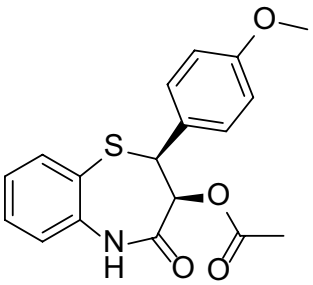

Fig. 3 Chemical structure of diltiazem EP impurity-B

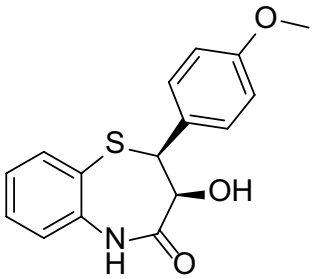

Fig. 5 Chemical structure of diltiazem EP impurity-E

Instrumentation

HPLC system (make: Waters) equipped with autosampler and quaternary gradient pump was used. The column compartment having temperature controlled and photodiode array detector (PDA) was used throughout the analysis. Chromatographic data were acquired using empower software.

Chromatographic conditions

A Zorbax RX C8 column (150 mm × 4.6 mm, 5 µm) (Agilent) was used as stationary phase maintained at 35 °C. The mobile phase involved a variable composition of buffer and organic solvents, mobile phase-A (mixture of 0.05 M sodium dihydrogen phosphate monohydrate buffer pH 3.0 and methanol in the ratio of 800:200 v/v, respectively) and mobile phase-B (containing acetonitrile). HPLC gradient program that was run is mentioned in Table 2.

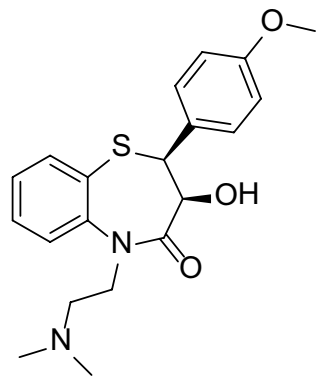

Fig. 6 Chemical structure of diltiazem EP impurity-F

Table 2 Mobile phase program for gradient elution

Time	Flow	Mobile phase-A (%)	Mobile phase-B (%)
0	1.0	90	10
3	1.0	90	10
13	1.0	75	25
40	1.0	70	30
41	1.0	90	10
50	1.0	90	10

Diluent

A mixture of 0.05 M sodium dihydrogen phosphate monohydrate buffer pH 3.0 and acetonitrile in the ratio of 60:40%v/v was used as a diluent.

Solution preparations

Standard solution

Solution containing 2.4 µg mL^{-1} of diltiazem standard was prepared in diluent.

Sample solution

Twenty tablets were accurately weighed, the average weight was determined, the tablets were crushed, and the crushed powder equivalent to 120 mg of diltiazem hydrochloride was transferred into a 100-mL volumetric flask. Seventy milliliters of diluent was added, sonicated for about 15 min with intermittent shaking and then diluted with diluent. It was filtered through 0.45-µm PVDF filter (concentration: 1200 µg mL^{-1}).

Forced degradation study

Multiple stressed samples were prepared as indicated in the following. They were chromatographed along with a non-stressed sample. %Degradation was calculated in terms of % total impurities (known and unknown impurities) and % degraded diltiazem peak along with mass balance reported in Table 3.

Acid degradation

Solution containing 1200 µg mL^{-1} of diltiazem hydrochloride was treated with 0.5 N HCl in a water bath maintained at 80 °C for 2 h. These solutions were neutralized as needed with 0.5 N NaOH.

Base degradation

Solution containing 1200 µg mL^{-1} of diltiazem hydrochloride was treated with 0.5 N NaOH in a water bath maintained at 80 °C for 2 h. These solutions were neutralized as needed with 0.5 N HCl.

Peroxide degradation

Solution containing 1200 µg mL^{-1} of diltiazem hydrochloride was treated with 5% w/v H_2O_2. This treated sample solution was kept for 24 h at room temperature.

Thermal degradation

Crushed tablets powder equivalent to 120 mg of diltiazem hydrochloride was transferred into a dry 100-mL volumetric flask, and these samples were exposed at 80 °C in an oven for 48 h.

Table 3 Degradation study data for diltiazem hydrochloride tablets

Stress condition	% Total Imp	% Assay Diltiazem	% Mass Balance	Purity Angle	Purity Threshold	Purity Flag
0.5 N HCl/80 ℃ in water bath for 2 h	7.21	90.17	97.4	0.070	0.278	No
0.5 N NaOH/80 ℃ in water bath for 2 h	6.88	90.92	97.8	0.068	0.283	No
5% H_2O_2 for 24 h at room temperature	3.45	97.45	100.9	0.051	0.243	No
Humidity/ (40 °C /75%RH) for 5 days	0.28	99.84	100.1	0.546	2.455	No
Thermal/80 °C/48 h	0.32	99.83	100.2	0.594	2.427	No
Photolytic/250 Wh/m^{2}/1.2 million Lux hours	0.26	99.86	100.1	0.586	2.444	No

Imp., impurity

Photolytic degradation

Crushed tablets powdered are directly exposed in a photolytic chamber for 1.2million Lux hours with energy not less than 200 Wh/m^2.

Humidity degradation

Crushed tablets powder equivalent to 120 mg of diltiazem hydrochloride was transferred into a dry 100-mL volumetric flask, and these tablets were exposed at 40 °C/75% RH for 5 days in a humidity chamber.

Results

Method validation

The developed reverse-phase HPLC analytical method was validated according to ICH guidelines with respect to specificity, accuracy, precision (method precision and intermediate precision), linearity, range and robustness.

Additional experiment of filter compatibility study conducted to prove the analysis results is not affected by the use of PVDF or nylon filter.

System suitability

The system suitability of test method was evaluated by injecting single injection of blank (diluent) solution and standard solution in six times.

USP plate count, USP tailing and %RSD of diltiazem peak area from six replicate injections of standard solution were evaluated.

The acceptance criteria defined from six replicate injections of standard solution are %RSD of diltiazem peak area should not be more than 5.0%, USP tailing for diltiazem peak should not be more than 2.0 and USP plate count for diltiazem peak should not be less than 5000.

Specificity

Peak purity results for the analyte in force degradation studies were determined with the PDA detector [39, 40] under optimized chromatographic conditions considered homogeneous (purity angle < purity threshold), indicating that no additional peaks were co-eluting with the analyte (diltiazem and its known impurity) and the specificity of the method. Resolution was achieved for all known, unknown impurities and degradants.

Stability of drug substance in analytical solution

The stability of the drug in the analytical solution was verified by preparing the sample solution as per the method and injecting at regular time intervals into proposed method at 7 °C temperature. On verifying the formation of additional peaks, it was found that no additional peaks were formed and there was no increase in the present known and unknown impurities by 0.04% level from its initial level to till 50 h, indicating that the sample solution is stable for about 50 h at 7 °C.

The stability of the standard solution was assessed by injecting the standard solution at various time intervals up to 59 h into the proposed method at 7 °C. %RSD of the diltiazem peak area was monitored from its initial level to 59 h and found less than 5.0% from its initial level, indicating that the standard solution is stable for about 59 h at 7 °C.

Filter paper compatibility study

Filter compatibility study was performed by analysis of duplicate preparation of spike sample (spiked with impurity-F) filtered with 0.45-µm PVDF filter and 0.45-µm nylon filter. Results were compared with centrifuged (unfiltered) spiked sample results. % absolute difference of %w/w impurity content and total impurities were evaluated. On verifying that the % absolute difference should not be more than 0.02% for individual impurity, % absolute difference should not be more than 0.04% for total impurities compared and no additional peak should be formed above limit of quantification level with unfiltered spike sample results. Filter compatibility results are reported in Table 4

Linearity and range

The test concentration for diltiazem hydrochloride is 1200 mg mL^{-1}. Consider the impurity tolerance limit level 0.3% with respect to test concentration of diltiazem hydrochloride. The response function was determined

Table 4 Filter paper compatibility results

Filter paper	Prep No.	Imp-F (%w/w)	SMU Imp (%w/w)	Total Imp (%w/w)
Centrifuge	1	0.35	0.02	0.41
Centrifuge	2	0.35	0.02	0.41
0.45-µm PVDF	1	0.35	0.02	0.41
0.45-µm PVDF	2	0.35	0.02	0.41
0.45-µm Nylon	1	0.35	0.02	0.41
0.45-µm Nylon	2	0.35	0.02	0.41
% Absolute difference (%w/w)				
Centrifuge	1	0.00	0.00	0.00
Centrifuge	2	0.00	0.00	0.00
0.45-µm PVDF	1	0.00	0.00	0.00
0.45-µm PVDF	2	0.00	0.00	0.00
0.45-µm Nylon	1	0.00	0.00	0.00
0.45-µm Nylon	2	0.00	0.00	0.00

Pre., preparation; SMU, single max unknown; Imp., impurity

Table 5 Regression statistics of linearity experimental results

Compound	Concentration µg mL^{-1}	Multiple R	Regression equation	F value	*P* value
Diltiazem hydrochloride	0.12 to 4.84	1.000	$y = 63810.96x + 1124.7$	11,398	< 0.05
EP Imp.-F	0.18 to 5.65	0.999	$y = 78198.34x + 178.13$	29,206	< 0.05

Table 6 Limit of quantification, detection and response factor (LOQ, LOD and RF)

Compound	LOQ		LOD		RF
	µg mL^{-1}	%w/w[a]	µg mL^{-1}	%w/w[a]	
EP impurity-F	0.18	0.015	0.06	0.005	0.82
Diltiazem hydrochloride	0.12	0.01	0.04	0.003	1.00

[a] %w/w calculated with respect to sample concentration (1200 µg mL$^{-1)}$

Table 7 Recovery for diltiazem hydrochloride

Levels	Preparation	% Recovery	Mean	%RSD
LOQ	1	113.0	112.3	0.5
	2	112.0		
	3	112.0		
50%	1	98.0	97.3	0.6
	2	97.0		
	3	97.0		
100%	1	98.5	98.5	0.0
	2	98.5		
	3	98.5		
150%	1	98.0	98.1	0.2
	2	98.3		
	3	98.0		

by preparing a standard solution of diltiazem hydrochloride and impurity-F at various concentration levels ranging from the lower limit of quantification to 150% of the impurity limit level.

The linearity graph of peak responses of the analytes relative to their corresponding concentrations was determined and found linear. The square of the residuals graph shows random pattern and passes the normal distribution test ($p < 0.05$) which prove the method is linear in the working concentration range.

The regression statistics of linearity experimental results are shown in Table 5.

Determination of limit of quantification and detection (LOQ and LOD)

The linearity carried out as indicated above was used for the determination of limit of quantification (LOQ), limit of detection (LOD) and residual standard deviation (σ).

And visual method based on signal-to-noise ratio was used for prediction of LOQ and LOD.

Precision was established for predicted level of LOQ and LOD. The LOQ and LOD results are given in Table 6.

$$\mathrm{LOQ} = 10\sigma/s$$
$$\mathrm{LOD} = 3.3\sigma/s$$

where σ = residual standard deviation of response and s = slope of the calibration curve.

Determination of response factor (RF) with linear calibration curve

Calibration curves for all components were constructed using the peak areas and analyte concentrations in the range reported in Table 5 by linear regression analysis. The linear regression equation containing the slope for all components is summarized in Table 5. The response factor (RF) was determined as the ratio of slope of the regression line of main drug component (diltiazem hydrochloride) to that for impurity and is listed in Table 6.

Accuracy

Accuracy was assessed by the simultaneous determination of analytes in solution prepared by standard addition method. The experiment was conducted by adding the known amount of impurity (impurity-F) in the test sample by considering the tolerance level, i.e., 0.3%w/w and diltiazem hydrochloride in the placebo (excipient) corresponding to four concentration levels at LOQ, 50%, 100% and 150% by considering the tolerance level, i.e., 0.3%w/w with respect to test sample concentration.

Triplicate sample at each level was prepared. The quantification of added impurity-F and diltiazem hydrochloride (%weight/weight) was calculated as per method by applying RF (response factor) of impurity.

The experimental finding has shown that approximately 80% to 120% of the recovery was obtained for the related compounds studied and %RSD for triplicate preparation of recovery results found less than 10%.

Therefore, based on the recovery experimental (Tables 7 and 8) the estimation of related substances

Table 8 Recovery for diltiazem EP impurity-F

Levels	Preparation	% Recovery	Mean	%RSD
LOQ	1	100.0	99.6	1.4
	2	98.0		
	3	100.7		
50%	1	99.3	100.0	0.7
	2	100.0		
	3	100.7		
100%	1	97.7	98.1	0.5
	2	98.0		
	3	98.7		
150%	1	98.2	98.2	0.2
	2	98.4		
	3	98.0		

that are prescribed in this report was shown to be accurate for its intended purpose and is adequate for routine analysis.

Method precision and ruggedness

According to the ICH (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use), ruggedness is considered as the method reproducibility and intermediate precision.

Method precision was evaluated by preparing six spike samples by spiking the known impurity (Impurity-F) at 0.3%w/w level with respect to test concentration (1200 µg mL^{-1}).

Intermediate precision was evaluated by different analysts on different HPLC systems on different columns on different days. Experiment was conducted same as method precision experiment by spiking the known impurities (impurity-F) at 0.3%w/w level with respect to test concentration.

Results (%w/w) were calculated for known, unknown and total impurities for method precision and intermediate precision experiment. %RSD was calculated for (%w/w) known, unknown and total impurities and found less than 10%

Overall %RSD for (%w/w) known, unknown and total impurities was calculated for method precision and intermediate precision experiment results ($n = 12$ results, six from method precision and six from intermediate precision) and found less than10.0%

The results for method precision and intermediate precision listed in Tables 9 and 10 reveal that the method has good reproducibility with acceptable precision.

Table 9 Comparison of method precision and intermediate precision results

Name	MP Imp-F	IP Imp-F	MP Unknown Imp. (SM)	IP Unknown Imp. (SM)
Spike sample-1	0.31	0.29	0.02	0.02
Spike sample-2	0.31	0.30	0.02	0.02
Spike sample-3	0.31	0.30	0.02	0.02
Spike sampel-4	0.31	0.30	0.02	0.02
Spike sample-5	0.30	0.30	0.02	0.02
Spike sample-6	0.30	0.30	0.02	0.02
Mean	0.31	0.30	0.02	0.02
%RSD	1.68	1.37	0.0	0.0
Overall Mean	030		0.02	
Overall %RSD	2.1		0.0	

MP, method precision; IP, intermediate precision; SM, single max

Robustness

The robustness of the method has been demonstrated by establishing the system suitability parameter by change in flow rate by ± 0.1 mL min^{-1}, change in column oven temperature by ± 5 °C, change in organic composition of mobile phase-A by ± 2% absolute and change in wavelength by ± 5 nm. The method was found robust by deliberate change in chromatographic conditions as mentioned above.

Discussion

The probable impurities of diltiazem hydrochloride are very similar to respective structure of drug substances. Diltiazem hydrochloride is freely soluble in water and methanol and practically soluble in acetonitrile [37].

The molecular structure (Figs. 1, 2, 3, 4, 5 and 6) shows that the diltiazem hydrochloride and its related

Table 10 Comparison of method precisions and intermediate precision results

Name	MP Total impurities	IP Total impurities
Spike sample-1	0.37	0.35
Spike sample-2	0.37	0.36
Spike sample-3	0.37	0.36
Spike sampel-4	0.37	0.36
Spike sample-5	0.36	0.36
Spike sample-6	0.36	0.36
Mean	0.37	0.36
%RSD	1.41	1.14
Overall Mean	0.36	
Overall %RSD	1.7	

MP, method precision; IP, intermediate precision

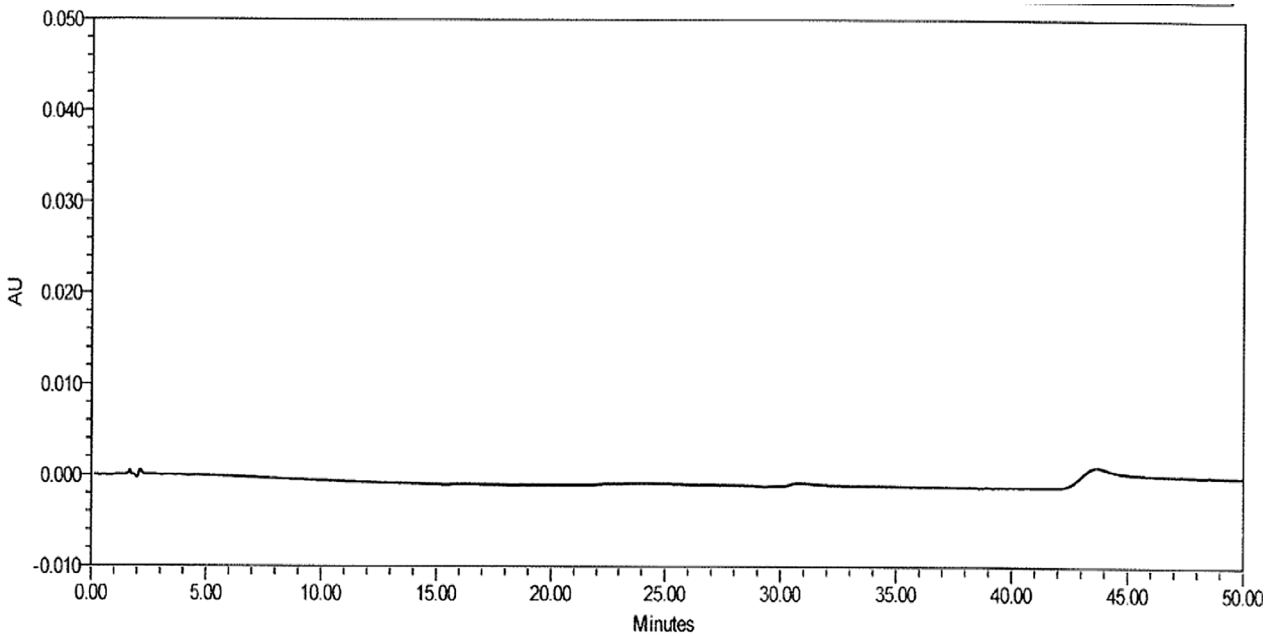

Fig. 7 Typical HPLC chromatogram of blank

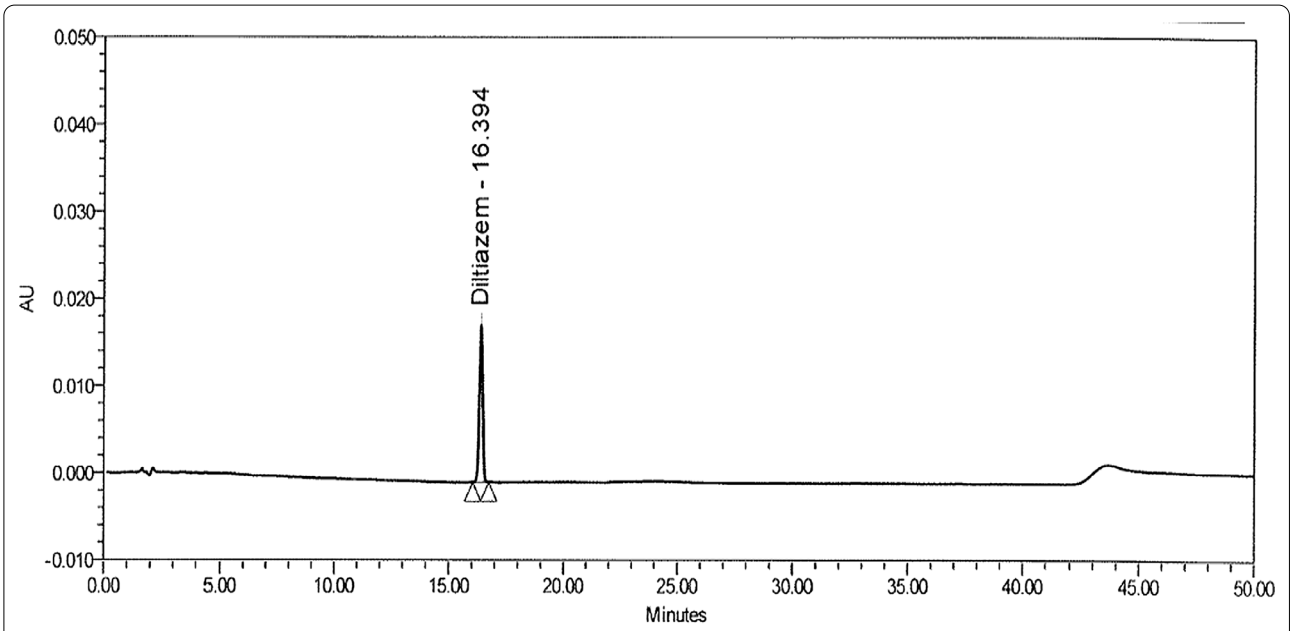

Fig. 8 Typical HPLC chromatogram of standard

compounds (impurity-A, impurity-B, impurity-C, impurity-E and impurity-F) are polar in nature due to the presence of amine group (–NH).

The objective of the method development is to separate the all known, unknown impurities and degradants among from each other as well as from main drug substances with short run time of analysis. Different columns consist of different stationary phases (RP-C_8 and RP-C_{18}), and different particle sizes of the column (3 µm and 5 µm) were also tested.

Considering diltiazem hydrochloride and their known related impurities polar in nature, the following mobile

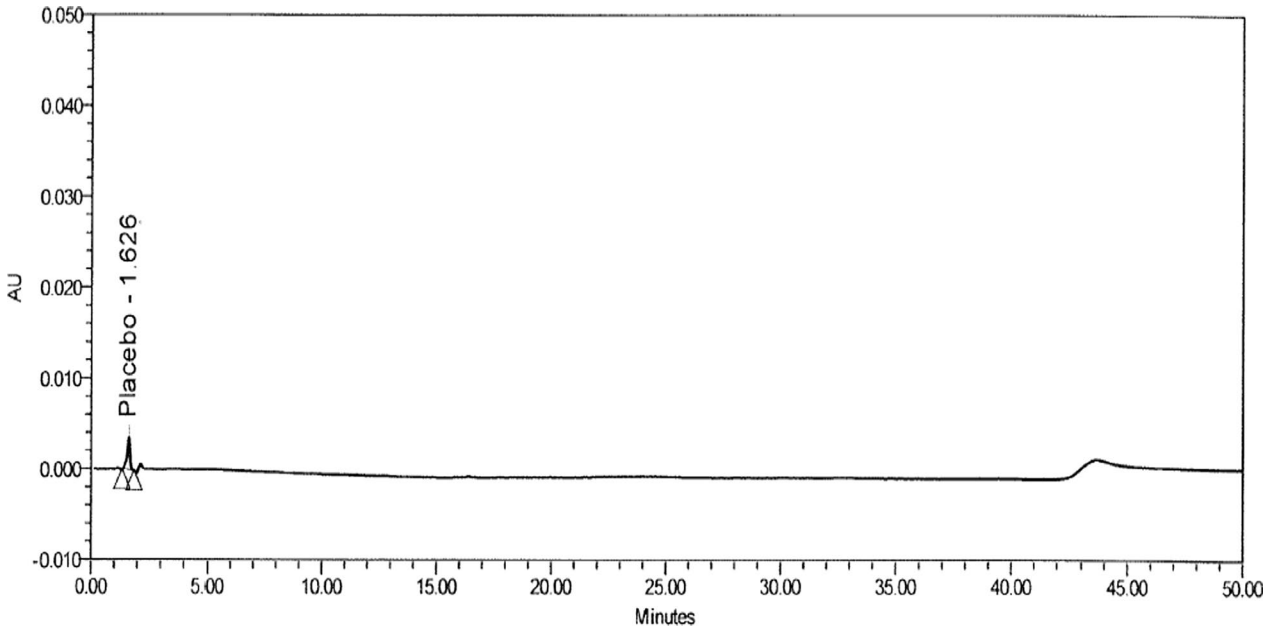

Fig. 9 Typical HPLC chromatogram of placebo solution

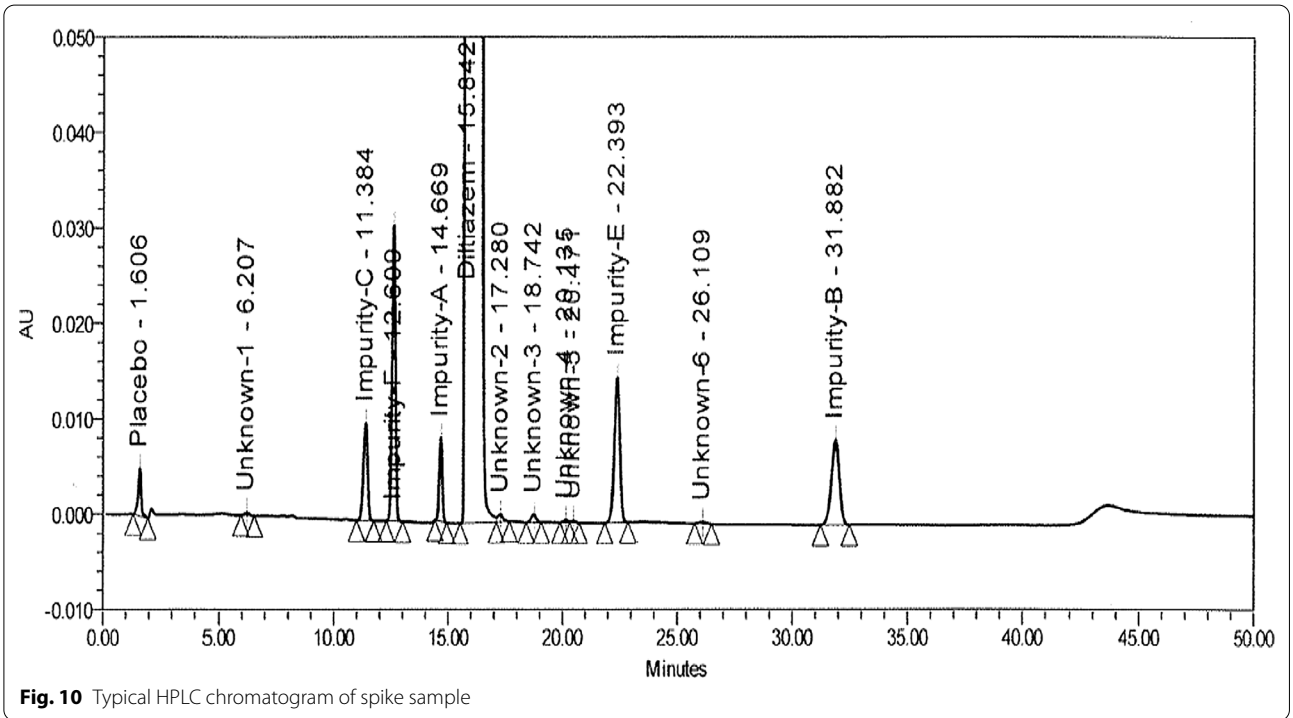

Fig. 10 Typical HPLC chromatogram of spike sample

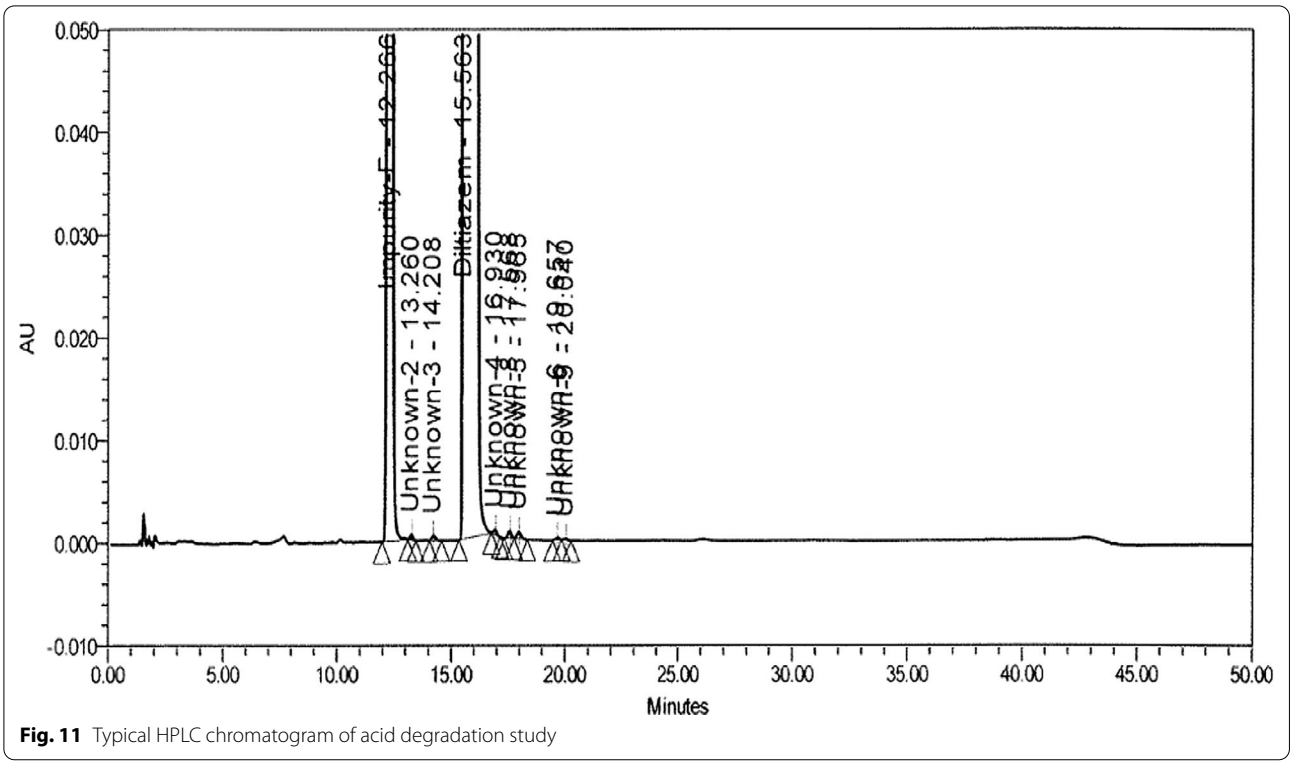

Fig. 11 Typical HPLC chromatogram of acid degradation study

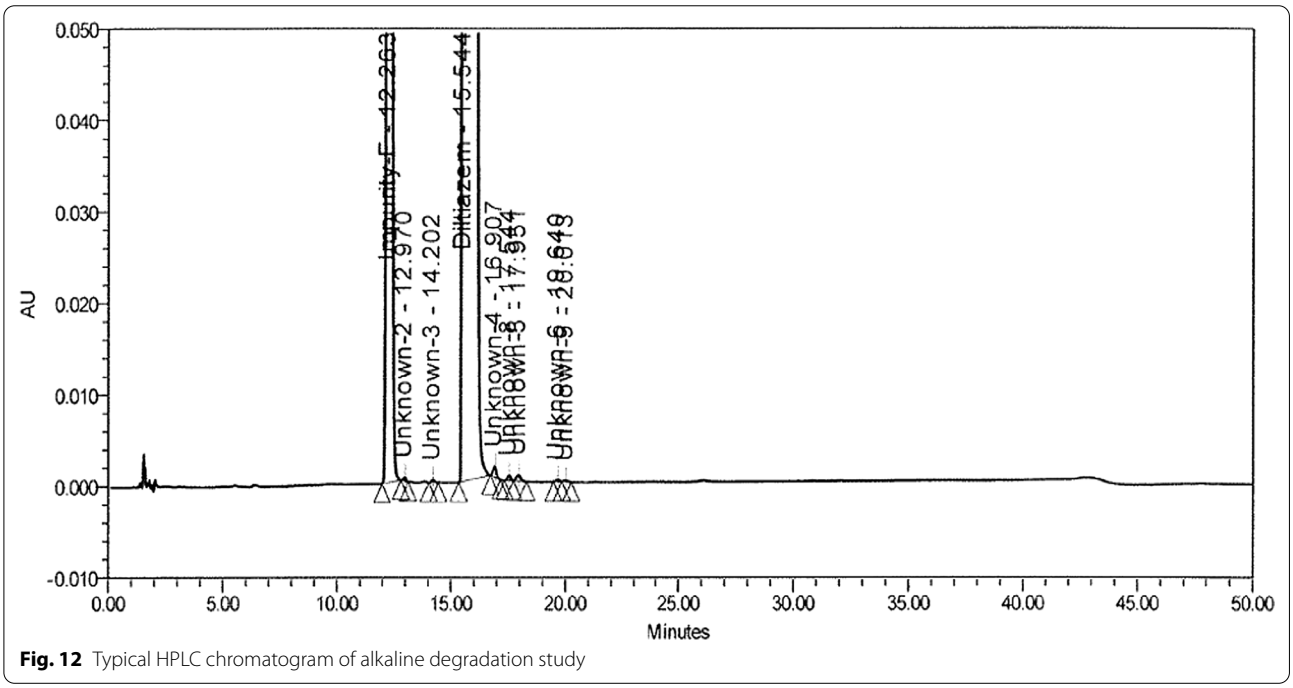

Fig. 12 Typical HPLC chromatogram of alkaline degradation study

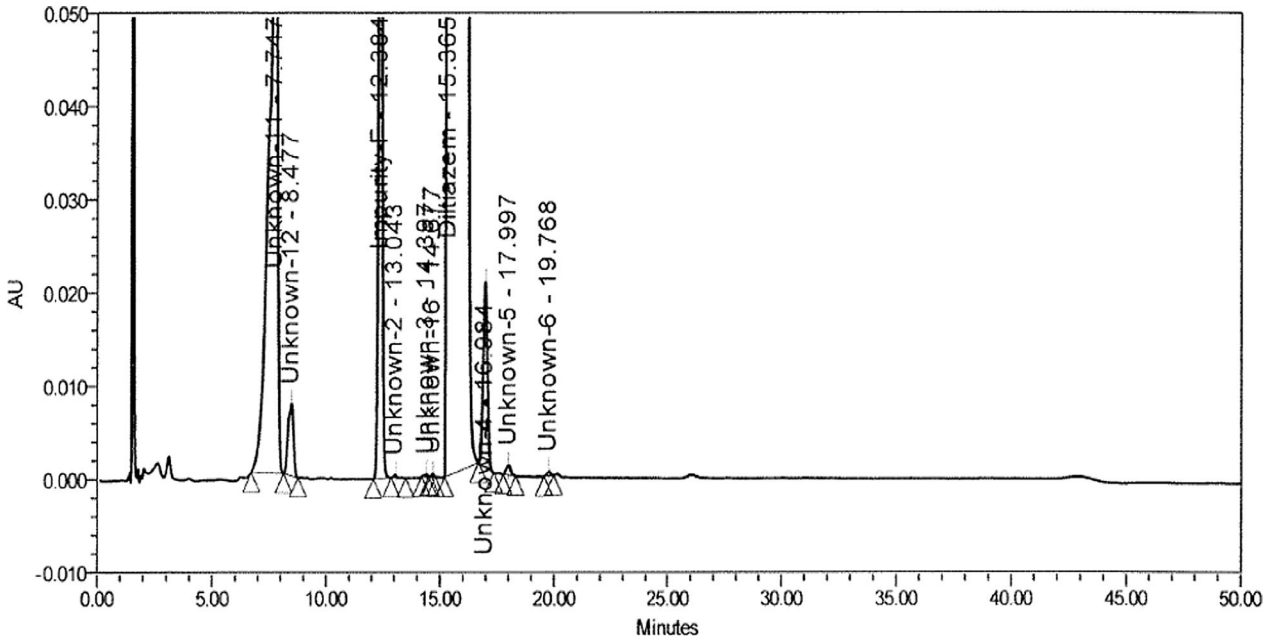

Fig. 13 Typical HPLC chromatogram of oxidative degradation study

phases with gradient elution attempts were done to separate all the impurities which are present and generated during stress studies:

(a) Trifluoroacetic acid buffer with methanol, (b) trifluoroacetic acid buffer with acetonitrile, (c) potassium dihydrogen phosphate buffer with methanol, (d) potassium dihydrogen buffer with acetonitrile, (e) sodium dihydrogen phosphate monohydrate buffer with methanol and (f) sodium dihydrogen phosphate monohydrate buffer with acetonitrile.

The pH of the above buffers varies from 2.8 to 4.0 during method development trials.

Selection of stationary phase: [7]

The poor resolution between diltiazem hydrochloride and diltiazem hydrochloride impurities (impurities-A, B, C, E and F) as well as broad peak shape for diltiazem hydrochloride observed, early elution (in void volume) of impurities implies that C_{18} stationary phase is not suitable for this application. Hence, C_8 stationary phase was chosen to improve resolution among the peaks and peak shape for diltiazem hydrochloride. The peak shape for diltiazem hydrochloride and resolution among all components improved with a Zorbax RX C8 column (150 mm × 4.6 mm, 5 µm).

Selection of mobile phase: [7]

Resolution among the known related impurities and unknown impurities of diltiazem hydrochloride was found poor using mobile phase with trifluoroacetic acetic acid buffer and potassium dihydrogen phosphate buffer. Broad peak shape for diltiazem hydrochloride and non-Gaussian peak shape were observed impurities with the use of the above buffer. The use of combination of sodium dihydrogen phosphate monohydrate buffer pH 3.0 with methanol gives the better resolution among the impurities and Gaussian peak shape for diltiazem and all impurities.

After an extensive study, the method has been finalized on a Zorbax RX C8 column (150 mm × 4.6 mm, 5 µm) using variable composition of mobile phase-A (mixture of 0.05 M sodium dihydrogen phosphate monohydrate buffer pH3.0 with methanol in the ratio 800:200 v/v, respectively) and mobile phase-B containing acetonitrile.

The mobile phase was at a flow rate of 1.0 mL min^{-1}, and column compartment temperature was kept at 35 °C. The detector response for all the components found maximum at 240 nm; hence, the typical chromatogram was recorded at this wavelength. The typical HPLC chromatograms for blank, standard, placebo and spike sample are represented in Figs. 7, 8, 9 and 10, respectively.

The typical HPLC chromatograms of acidic, alkaline and photolytic stressed conditions (force degradation) sample are represented in Figs. 11, 12 and 13, respectiv ely.

In acid degradation, alkaline degradation (Figs. 11 and 12, respectively), it was observed that the known impurity-F found major degradation impurity. In oxidative

degradation (Fig. 13), unknown impurity at 0.55 RRT found major degradation impurity and known impurity-F increased (1.05%w/w) significantly from its initial level (0.04%w/w).

Conclusion

Analytical method validation experiment results revealed that the newly developed analytical method is linear, accurate, specific and precise in the established working concentration range. Forced degradation study and specificity experiment results with mass balance prove the stability-indicating nature of the method and separate all known, unknown impurities and degradants from each other as well as from main drug component (diltiazem hydrochloride).

The method is robust to change in flow rate, column oven temperature, change in wavelength and change in organic composition of mobile phase.

Hence, this novel analytical method can be intended for routine analysis as well as to monitor the known, unknown and degradant profile of diltiazem hydrochloride pharmaceutical dosage form (tablets).

Abbreviations

LOQ: Limit of Quantification; LOD: Limit of Detection; RRT: Relative Retention Time; EP: European Pharmacopoeia; HPLC: High-Performance Chromatography; MS: Mass Spectrophotometer; RH: Relative Humidity.

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Authors' contributions

NM conceptualized, designed and executed the current research work. SD and MF supervised the work. All the authors read and approved the final manuscript.

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Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

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Competing interests

The authors declare that they have no competing interest.

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