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QUANTIFICATION AND METHOD VALIDATION OF DICYCLOHEXYLUREA CONTENT IN FOSAPREPITANT DIMEGLUMINE DRUG SUBSTANCE BY REVERSE PHASE HPLC

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Keywords:

Fosaprepitant dimeglumine, Dicylohexylurea, Method development, Method validation and HPLC

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ABSTRACT: A simple, sensitive, and rapid reverse phase High-Pressure Liquid Chromatography (HPLC) method was developed and validated for the quantification of residual Dicyclohexylurea in Fosaprepitant dimeglumine drug substance at trace level. The method was optimized on Zorbax Eclipse XDB-C8, 250 mm × 4.6 mm, 5µm with column oven temperature maintaining at 35 °C with flow rate at 0.7 ml/min. Mobile Phase was prepared by a mixture of phosphate buffer pH 7.0 (0.68 g of Potassium dihydrogen orthophosphate in 1000 ml of water) and Acetonitrile in the ratio of 53:47 v/v), the injection volume is 20 µl, the detector wavelength is 205 nm. The developed, optimized method was further validated in accordance with ICH, and the method is found to be specific, sensitive, accurate, and precise. The Limit of detection (LOD) and Limit of quantification (LOQ) for dicyclohexylurea are 20 µg/g and 60 µg/g, respectively. The method validation experimental results are discussed in detail in this research paper.

INTRODUCTION: The chemical name of Fosaprepitant dimeglumine is 1-Deoxy-1-(Methyl-amino)-D-Glucitol[3-[[(2*R*, 3*S*)- 2-[(1*R*)-1-[3, 5-bis (trifluoromethyl) phenyl] ethoxy]- 3- (4- fluoro-phenyl)- 4- morpho- linyl]methyl]-2, 5-dihydro-5-oxo-1*H*-1, 2, -triazol-1-yl]phosphonate (2:1), the molecular formula is $C_{23}H_{22}F_7N_4O_6P.2(C_7H_{17}NO_5)$ and molecular weight is 1004.83. The US Food and Drug Administration (USFDA) has approved the first single-dose intravenous NK1 receptor antagonist, fosaprepitant dimeglumine (Emend), for the treatment of nausea and vomiting that can accompany the use of moderately and highly emetogenic chemotherapy [1].

The drug is approved in combination with other antiemetic's and the potential for drug interaction with aprepitant and fosaprepitant should be considered when selecting antiemetic therapy [2]. Fosaprepitant is an intravenous prodrug of aprepitant that offers a new alternative to patients with Chemotherapy-induced nausea and vomiting (CINV). The use of aprepitant and fosaprepitant has been a significant step in reducing nausea and vomiting associated with chemotherapy administration. Over the past two years, several consensus statements and guidelines for the prevention of CINV in pediatric patients have been updated to include their use [3].

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Currently, this drug can substitute oral aprepitant in day 1 of a 3-day regimen. Fosaprepitant dimeglumine is a phosphorylated prodrug, and this drug is quickly converted to aprepitant, which is also known oral selective neurokinnin-I receptor antagonist approved [4].

In the recent years, Kusick and *et al*., provided a preliminary report of safety data on fosaprepitant use in 20 children, who received 87 doses for CINV prophylaxis and no other adverse reactions were noted [5].

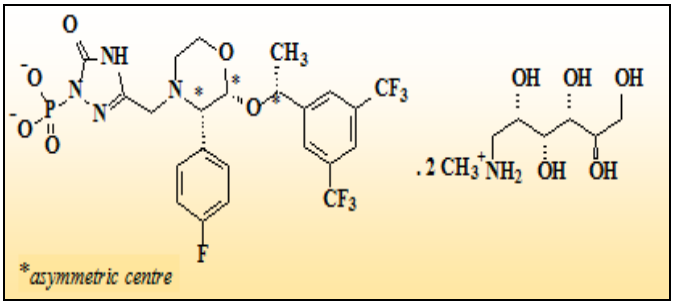


FIG. 1: CHEMICAL STRUCTURE OF FOSAPREPITANT DIMEGLUMINE

The USFDA approved dose of Fosaprepitant is 115 mg for European, Australian authorities. This drug may be a useful parenteral alternative to oral aprepitant. The brand name of Fosaprepitant dimeglumine is EMEND IV[6] for US and IVEMEND [7] in the form of lyophilized powder in single-dose vial for reconstitution, and each vial contains Fosaprepitant dimeglumine equivalent to 150 mg of Fosaprepitant, which corresponds to 130.5 mg of aprepitant. The reconstitution and dilution 1 ml of solution contain 1 mg of FP (1 mg/ml). Fosaprepitant dimeglumine chemical structure has been shown in **Fig. 1**.

The process for the preparation of fosaprepitant dimeglumine by reacting aprepitant compound with tetrabenzyl pyrophosphate in the presence of a sodium hexamethyldisilazane (NaHMDS) base gives Dibenzyl Fosaprepitant (Key intermediate for the preparation of Fosaprepitant dimeglumine), where Tetrabenzyl pyrophosphate preparation by Dibenzyl Phosapate treated with 1,3-dicyclohexylcarbodiimide (DCC) in the presence of Isopropyl acetate. Although the dibenzyl phosphate is not completely soluble in isopropyl acetate, it is apparently sufficiently soluble to allow formation of tetrabenzyl pyro-phosphate, which remains in solution while a slurry of 1,3-dicyclohexylurea (DCU) forms as a white precipitate [8], filtration process applied in the process to remove this excess of DCU. The process scheme has been shown in **Fig. 2**. In general, residual quantities of impurities may contain surplus to the final drug substance.

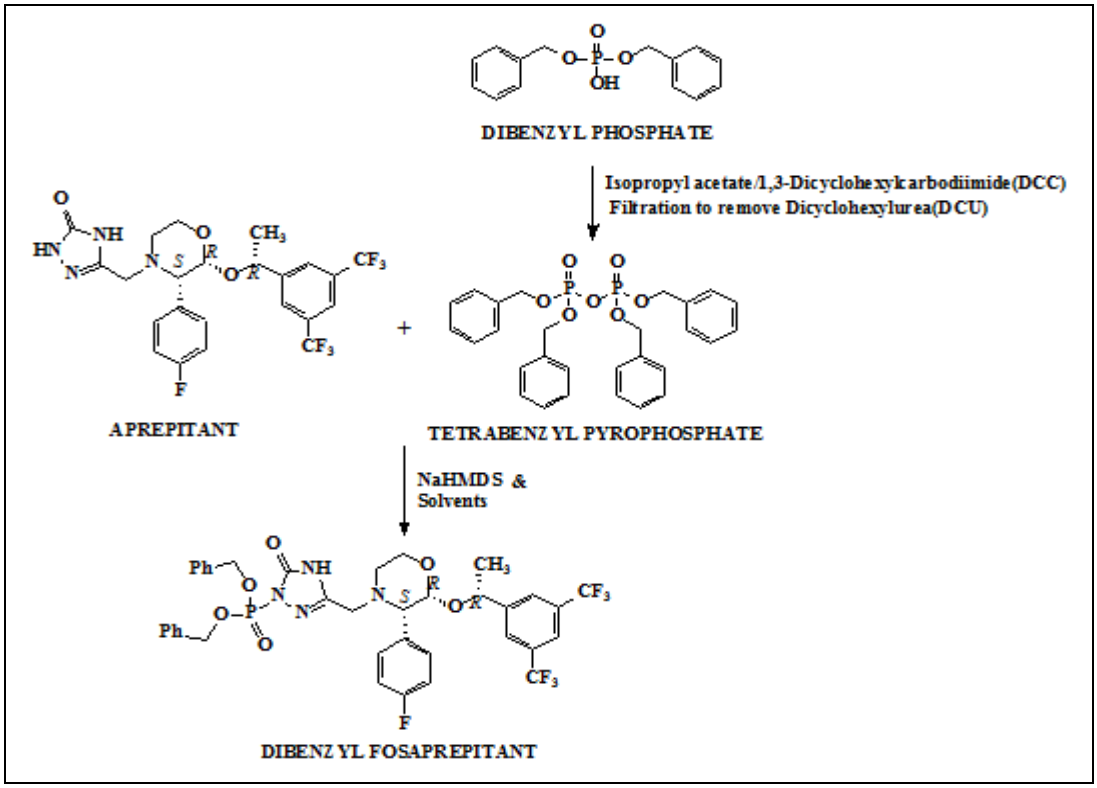


FIG. 2: ORIGIN OF DICYCLOHEXYLUREA IN THE PREPARATION OF DIBENZYL FOSAPREPITANT

In view of this, control of DCU is required in Fosaprepitant dimeglumine samples and monitored with a lower level limit not more than 500µg/g. The specified limit is below lower than the accepted limit as per ICH[9]. The best of our knowledge, determination of DCU by HPLC in Fosaprepitant dimeglumine drug substance has not been reported in literature to date. Hence, we aimed to develop an RP-HPLC method for the determination and validation of DCU in Fosaprepitant dimeglumine as per ICH and FDA guidelines [10-11]. The formation of Dicyclohexylurea (DCU) and chemical structure, as shown in **Fig. 3**.

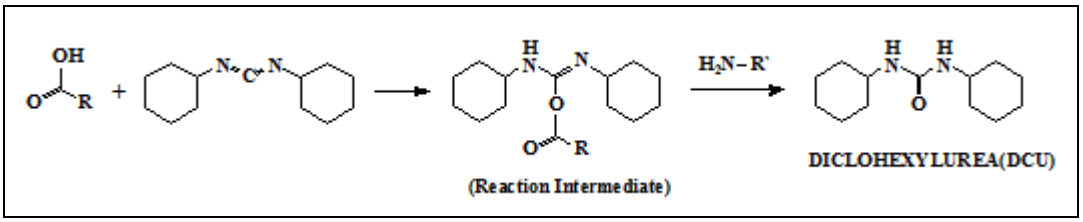


FIG. 3: FORMATION OF DICYCLOHEXYLUREA FROM DCC

EXPERIMENTAL:

Chemicals, Reagents and Samples: Fosaprepitant dimeglumine drug substance, its related substances, and Dicyclohexylurea were procured from APL Research Centre-II (A division of Aurobindo Pharma Ltd., Hyderabad, India). Potassium dihydrogen orthophosphate (Analytical grade), Acetonitrile (HPLC grade), Methanol (Gradient grade), were procured from Merck, India, and highly pure milli-Q water was obtained by using millipore purification system.

Instrumentation and Chromatographic Conditions: Chromatographic separations were performed on HPLC (High-Performance Liquid Chromatography) system with Alliance-waters e2695 separation module with 2998 PDA detector using Empower software. Buffer was prepared by dissolving 0.68 g of Potassium dihydrogen ortho-phosphate in 1000 ml of water and adjusted to pH 7.0 with potassium hydroxide solution; the further mobile phase was prepared by using buffer and acetonitrile in the ratio of 53: 47% w/v. Diluent was prepared by a degassed mixture of methanol and water in the ratio of 1:1 v/v.

The analysis was carried out on Zorbax Eclipse XDB-C8, 250 mm × 4.6 mm, 5 µm particle diameter column (Make: Agilent), [A stainless steel column 250 mm long, 4.6 mm internal diameter filled with octyl silane chemically bonded to porous silica particles of 5 µm diameter] maintained at temperature 35 °C and the pump was in isocratic mode with flow rate 0.7 ml/min. The wavelength detection at 205 nm, the injection volume was 20 µl. The run time for the standard was kept as 25 min with an initial gradient ratio, and the sample was 35 min, all these chromatographic conditions, dicyclohexylurea peak elutes at about 13 min.

Preparation of Solutions:

Standard Solution: Accurately weigh and transfer about 20 mg of Dicyclohexyl urea reference standard into a 100 ml clean, dry volumetric flask, add 70 ml of methanol and sonicate to dissolve. Makeup to volume with methanol. Dilute 5 ml of this solution to 100 ml with diluent. Further, dilute 5 ml of this solution to 50 ml with diluent Concentration about 0.001 mg/ml).

Sample Solution: Accurately weigh and transfer about 50 mg of sample into a 25 ml clean, dry volumetric flask, add 15 ml of diluent and sonicate to dissolve. Makeup to volume with diluents (Concentration about 2 mg/ml).

System Suitability Criteria: The column efficiency as determined from the Dicyclohexyl urea peak is not less than 8000 USP plate count, and USP tailing for the same peak is not more than 1.5 from Dicyclohexyl urea 20 µl standard solution chromatogram.

RESULTS AND DISCUSSION:

Method Development: To present this research work, we have aimed this study for better development from RP-HPLC and is to develop simple, sensitive and rapid, and robust chromatographic method which can separate analyte peak from Fosaprepitant dimeglumine. We have tried different concentrations of pH buffers

from lower to the higher side, pH range between 3.0 and 5.0 buffers, dicyclohexylurea peak is eluted at void volumes, and hence, pH 7.0 buffer was selected. Initial trials of the isocratic mode were given good separation from drug substance peak; the isocratic pump mode has been finalized based on the adjustment of flow rate. The main target of this research work is to avoid drug substance-related substances interference to analyte peak, to achieve this target, stationary phase selection is very critical, after so many trails of using different column dimensions, we have selected Zorbax Eclipse XDB-C8, 250 mm × 4.6 mm, 5 µm particle diameter column (Make: Agilent), [A stainless steel column 250 mm long, 4.6 mm internal diameter filled with octyl silane chemically bonded to porous silica particles of 5 µm diameter]. By using this column and chromatographic parameters, we have validated the method for suitability.

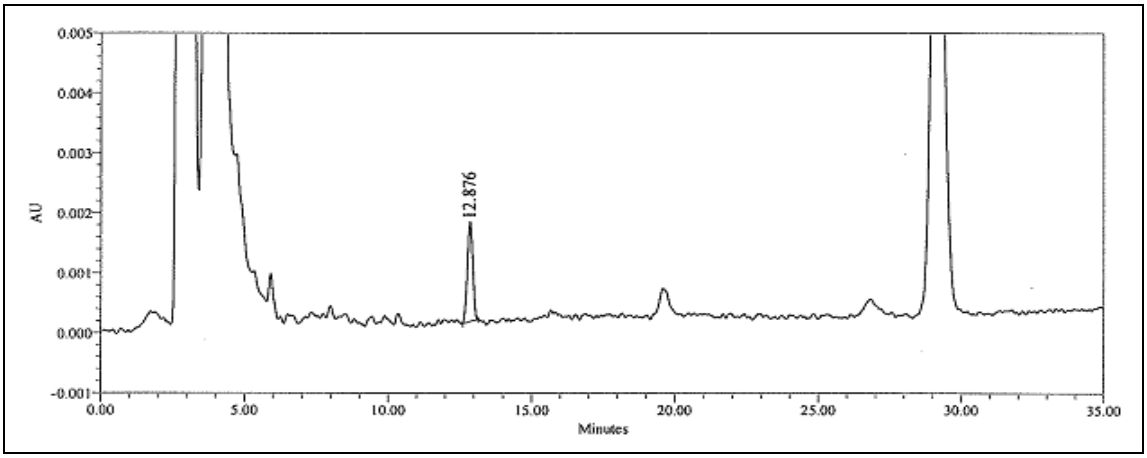
Method Validation: The developed and optimized method was then validated for its specificity, linearity, LOD and LOQ, accuracy, the stability of solutions and precision to demonstrate that the method is suitable for its intended use per regular sample analysis to quantify the levels of Dicylcohexylurea in Fosaprepitant dimeglumine drug substance.

Specificity: Specificity of the method is demonstrated in terms of spectral as well as peak purity data of the drug, and its related substances are present in Fosaprepitant dimeglumine drug substance. The analyte peak passed the peak purity test. The solutions of diluent, Fosaprepitant dimeglumine drug substance, Fosaprepitant dimeglumine drug substance spiked with Dicyclohexyl-urea (control sample) and Fosaprepitant dimeglumine drug substance spiked with all known related substances including Dicyclohexyl urea (spiked sample) were injected to confirm any co-elution with Dicyclohexyl urea peak from any known related substances. Peak purity for Dicyclohexyl urea was established by using waters empower software. From the integrated HPLC chromatograms, no peak is observed at the retention time of Dicyclohexyl urea peak in the diluent chromatogram. Further, the peak purity data of Dicyclohexyl urea from the control sample and spiked sample indicated that the peaks were homogeneous and had no co-eluting peaks. Based on these observations, it can be concluded that there is no interference due to listed known related substances for the determination of Dicyclohexyl urea content in Fosaprepitant dimeglumine drug substance. The typical HPLC chromatograms of Fosaprepitant dimeglumine spiked with Dicyclohexylurea and Fosaprepitant dimeglumine spiked with all known related substances including Dicyclohexylurea are shown in **Fig. 4**. The specificity experiment data is given in **Table 1**.

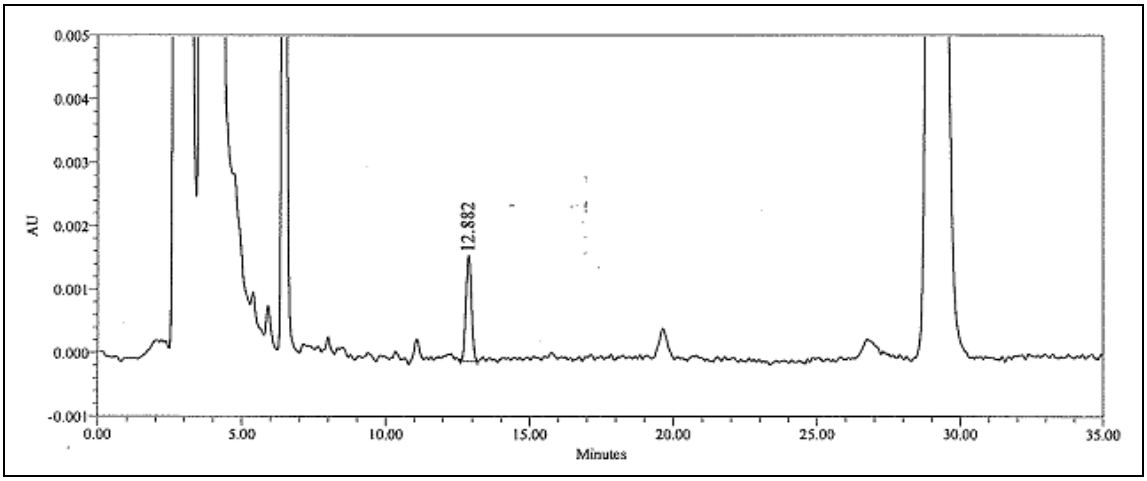
TABLE 1: SPECIFICITY EXPERIMENTS DATA

Sample	Dicyclohexylurea Retention Time (min)	Peak Purity	
		Purity angle	Purity threshold
Control sample	12.876	2.703	8.855
Spiked sample	12.882	2.567	9.906

Based on this experimental data, the peak purity data of Dicyclohexylurea from the control sample and spiked sample indicated that the peaks were homogeneous and had no co-eluting peaks.



CONTROL SAMPLE (SPIKED WITH DICYLOHEXYLUREA)



SPIKED SAMPLE (SPIKED WITH DICYLOHEXYLUREA WITH KNOWN RELATED SUBSTANCES)
FIG. 4: TYPICAL HPLC CHROMATOGRAMS OF SPECIFICITY EXPERIMENT

Hence, it can be concluded that there is no interference due to listed known related substances for the determination of Dicyclohexylurea content in Fosaprepitant dimeglumine drug substance.

LOD and LOQ: The method sensitivity was established by determining the limit of detection (LOD) and limit of quantitation (LOQ). The LOD/ LOQ values of Dicyclohexylurea were determined from based on signal to noise ratio data. The predicted concentrations of LOD and LOQ for Dicyclohexylurea were verified for precision by preparing the solutions containing Dicyclohexylurea at about predicted concentrations. Each of these solutions six times, injected into the HPLC.

Linearity: The limit of detection and limit of quantitation values of Dicyclohexyl urea were determined from signal to noise ratio of the analyte. The predicted concentrations of Limit of detection and Limit of quantitation for Dicyclohexyl urea were verified for precision by preparing the solutions containing Dicyclohexyl urea at about these predicted concentrations. Injected each solution six times into the HPLC by following the test method conditions. The LOD and LOQ experiments data is shown in **Table 2**. Further, the linearity of the method was checked by preparing solutions at nine concentration levels from LOQ to 150% of specification level (0.05%) by prepared using of Dicyclohexylurea standard solution, and each solution was injected into HPLC. Linearity was established by using concentration (µg/ml) on X-axis, area on Y-axis, and calculated statistical values like slope, intercept, residual sum of squares, and correlation coefficient.

TABLE 2: LOD/LOQ AND LINEARITY EXPERIMENTS DATA

Injection	Area of Dicyclohexyl urea	
	LOD	LOQ
1	930	2924
2	924	2916
3	936	2948
4	910	2871
5	960	2909
6	926	2900
Statistical analysis		
Mean	931	2911
SD	17	26
% RSD	1.8	0.9
Concentration levels		
Conc. (µg/g)	20	60

Accuracy: Accuracy of the method was performed by recovery experiments using standard addition technique. Sample solutions were prepared in triplicate by spiking Dicyclohexylurea at levels of LOQ to 150% of specification limit as per the test method and injected each solution into HPLC as per methodology, and the percentage recoveries were calculated. The fully validated recovery results are shown in **Table 3**.

Precision: System precision was confirmed by preparing the standard solution of Dicyclohexyl-urea as per test procedure and analyzed by injecting six replicates. Method precision experiments established by preparing six sample solutions individually using a one batch of Fosaprepitant dimeglumine drug substance spiked with Dicyclo-hexylurea at specification level and determined the Dicyclohexylurea content by HPLC. Achieved results like %RSD and a 95% confidence interval for six determinations are summarized in **Table 4**.

TABLE 3: ACCURACY DATA

% Level/Sample ID	Amount Added (µg/g)	Amount Found (µg/g)	Recovery (%)	Average
LOQ Level Sample - 1	59.64	57.93	97.1	96.1
LOQ Level Sample - 2	59.52	56.13	94.3	
LOQ Level Sample - 3	59.17	57.34	96.9	
50 % Level Sample 1	248	252	101.6	99.5
50 % Level Sample 2	250	245	98.0	
50 % Level Sample 3	255	249	97.6	
100% Level Sample 1	500	500	100.0	
100% Level Sample 2	495	502	101.4	
100% Level Sample 3	497	488	98.1	
150% Level Sample 1	745	751	100.8	
150% Level Sample 2	755	749	99.2	
150% Level Sample 3	756	748	98.9	

TABLE 4: PRECISION DATA

	Injection ID	Dicyclohexylurea area	Statistical Analysis		
System Precision	1	23798			
	2	23748			
	3	23883	Mean	23836	
	4	23821	SD	70	
	5	23949	% RSD	0.3	
	6	23815	95% Confidence Interval (±)	73	
	Sample	**Dicyclohexylurea (%w/w)**	**Statistical Analysis**		
Method Precision	1	489			
	2	494			
	3	493	Mean	494	
	4	495	SD	3	
	5	499	% RSD	0.6	
	6	492	95% Confidence Interval (±)	3	
	Sample	**Dicyclohexylurea (%w/w)**	**Statistical Analysis**		
Intermediate Precision	1	546			
	2	547		For ruggedness	overall
	3	552	Mean	549	521
	4	548	SD	2	29
	5	551	% RSD	0.4	5.6
	6	549	95% Confidence Interval (±)	2	18

Solution Stability: For the determination of stability of the standard and sample solutions, standard solution and sample solution spiked with Dicyclohexylurea at specification level were prepared as per test methodology and analyzed initially and at different time intervals by keeping the solution at room temperature (25° ± 2 °C). The % difference in the peak area obtained at initial and after 13 h time interval was found to be less than 8.2 for a standard solution and 2.0 for sample solution at room temperature (25° ± 2 °C). Based on data, it was concluded that the standard solution is stable at least 13 h, and sample solution is stable at least for 15 h at 25° ± 2 °C temperature. The summarized results are shown in **Table 5**.

TABLE 5: STABILITY OF SOLUTIONS EXPERIMENT DATA

		Dicyclohexylurea	% Difference
Standard (at 25°±2°C)	Initial	24356	2.7
	After 15 h	25018	
Sample (at 25°±2°C)	Initial	25895	0.5
	After 15 h	25762	

CONCLUSION: The HPLC chromatography method was developed, optimized, and validated for the determination of residual Dicyclohexylurea content in Fosaprepitant dimeglumine drug substance and the results of various validation parameters proved that the method is specific, sensitive, precise and accurate and the method can be introduced into routine testing.

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CONFLICTS OF INTEREST: The authors declare that there is no conflict of interest regarding this publication of the article.

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