VALIDATED SPECTROFLUORIMETRIC METHOD FOR ESTIMATION OF IDELALISIB IN BULK AND IN FORMULATION**

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Idelalisib is a phosphatidylinositol 3-kinase delta inhibitor approved by the USFDA and EMA for the treatment of lympholytic lymphoma, B-cell non-Hodgkin lymphoma and lymphocytic lymphoma. The present report describes the validation of simple, rapid, sensitive, and cost-effective spectrofluorimetric methods based on the native fluorescence of the drug in an acidic medium. Fluorescence characteristics of the drug were found to significantly differ in absolute ethanol (λ_{ex} = 330 and λ_{em} = 595 nm) and HCl (λ_{ex} = 270 and λ_{em} = 350 nm) and both methods were validated as per ICH guidelines. The two methods were extremely sensitive, precise and accurate demonstrating excellent linearity in concentration ranges from 0.1–2.0 μg/mL (absolute ethanol) and 0.1–20 μg/mL (HCl). The LOD and LOQ values were found to be 0.015 and 0.045 μg/mL (ethanol) and 0.1615 and 0.4894 μg/mL (HCl). The proposed methods were used to quantify the drug in its marketed tablet formulation with good recoveries, suggesting their applicability to routine analysis of the drug in bulk as well as in formulation.

Keywords: *spectrofluorimetric method, bulk, formulation, idelalisib.*

СПЕКТРОФЛУОРИМЕТРИЧЕСКИЙ МЕТОД ОПРЕДЕЛЕНИЯ ИДЕЛАЛИСИБА В НЕРАСФАСОВАННОМ ВИДЕ И ФАРМАЦЕВТИЧЕСКИХ СОСТАВАХ

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Иделалисиб представляет собой дельта-ингибитор фосфатидилинозитол-3-киназы, одобренный USFDA и EMA для лечения лимфолитической лимфомы, В-клеточной неходжкинской лимфомы и лимфоцитарной лимфомы. Описаны простые, чувствительные и экономичные спектрофлуориметрические методы, основанные на нативной флуоресценции лекарственного средства в кислой среде. Обнаружено, что характеристики флуоресценции препарата значительно различаются в абсолютном этаноле (λ_{ex} = 330 и λ_{em} = 595 нм) и HCl (λ_{ex} = 270 и λ_{em} = 350 нм). Методы валидированы в соответствии с рекомендациями ICH, демонстрируют превосходную линейность в диапазонах концентраций 0.1–2.0 мкг/мл (абсолютный этанол) и 0.1–20 мкг/мл (HCl), LOD и LOQ составляют 0.015 и 0.045 мкг/мл (этанол) и 0.1615 и 0.4894 мкг/мл (HCl). Предложенные методы использованы для количественного определения лекарственного средства в таблетированной форме с хорошим выходом, что позволяет предположить их применимость для рутинного анализа лекарственного средства в нерасфасованном виде.

Ключевые слова: *спектрофлуориметрический метод, объем, рецептура, иделалисиб.*

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Introduction. Idelalisib [(5-fluoro-3-phenyl)-2-[(1*S*)-1-(9*H*-purin-6ylamino)propyl]quinazolin-4(3*H*)-one) is an oral phosphatidylinositol 3-kinase (PI3K) delta inhibitor [1, 2] indicated for the treatment of patients with relapsed follicular B-cell non-Hodgkin lymphoma and relapsed small lymphocytic lymphoma [3]. Idelalisib was the first agent in the class of isoform-specific inhibitors to receive regulatory approval from the US Food and Drug Administration (USFDA) and European Medicine Agency (EMA) for the treatment of lympholytic leukemia as a monotherapy or in combination with other PI3K inhibitors like rituximab, bendamustine and ofatumumab [3–6]. Very few reports could be retrieved from the literature relating to the development and validation of analytical or stability-indicating methods for idelalisib. The few documented analytical methods reported for the drug are based on chromatographic procedures. The isolation and identification of degradation impurities in idelalisib have been reported by HPLC by Bommuluri et al. [7]. The majority of the reported analytical methods for idelalisib are performed in plasma samples in animals and man. These include the method development and validation for the estimation of the drug in rabbit plasma, which has been reported by HPLC [8], and the quantification of idelalisib in human plasma by UPLC coupled to mass spectrometry in negative ionization mode [9]. A dried blood spot (DBS) assay method for the drug has been reported based on LC-MS/MS, operated under a multiple reaction monitoring mode [10].

Simultaneous determination of idelalisib has been performed along with its metabolite GS-563117 in dog plasma by LC-MS/MS and the method has been applied to a pharmacokinetic study [11]. Simultaneous quantification of idelalisib in the presence of two other drugs, fludarabine and lenalidomide, has been reported in rat plasma by using HPLC coupled with heated electrospray ionization tandem spectroscopy [12]. Another validated LC-MS/MS method has been reported for the simultaneous quantitation of idelalisib in mouse plasma in the presence of two other PI3K Inhibitors, copanlisib, and duvelisib, followed by its application to a pharmacokinetic study in mice [13]. To date, there is no report on the development and validation of a spectrofluorimetric method for this drug, although its structure shows the presence of potential fluorophoric features. Spectrofluorimetric methods have several advantages over simple UV-visible spectrophotometric methods, including much higher sensitivity (nearly 100 times; comparable with HPLC methods) and selectivity (attributed to an exclusive combination of excitation and emission wavelengths). When compared with the chromatographic methods, spectrofluorimetric methods are more cost-effective and convenient; hence, this highly sensitive, economical and simple technique can be particularly useful for the development of analytical methods for the estimation of drugs present in extremely low amounts. Considering the amenability of idelalisib molecule to spectrofluorimetric estimation, the present study was envisaged to develop simple, rapid, and reproducible spectrofluorimetric methods for the quantification of idelalisib in bulk and in its marketed tablet formulation (Zydelig^{TM}, manufactured by Gilead Sciences, Inc.). The developed methods were validated with respect to various parameters outlined in the ICH guideline Q2 (R1) [14].

Experiment. All chemicals and materials were of analytical grade purchased from Merck India Pvt. Ltd., Mumbai and all solutions were freshly prepared in triple distilled water prepared using Milli-Q plus purification system, Millipore (Bradford, USA). Hydrochloric acid, hydrogen peroxide (30%), sodium hydroxide and ethanol, used for sample preparation, were of AR grade and were procured commercially from Merck India Pvt. Ltd., Mumbai. A tablet formulation containing idelalisib (label claim 100 mg; Zydelig^{TM}, manufactured by Gilead Sciences, Inc.; Batch no. 61958) was purchased from the local market.

All the glassware, including volumetric flasks, pipettes, measuring cylinders, beakers, test tubes and round bottom flasks were of class A grade purchased from Borosil. Absorption and emission spectra were recorded using a Hitachi spectrofluorimeter F 2500 equipped with a 150 W xenon lamp in the self-deozonating lamp housing, grating excitation and emission monochromators, 1-cm pathlength cells, with a scanning speed of 1,500 nm/min, 10-mm matched quartz cells and a resolution of 2.5 nm. Melting point apparatus (model T0603160; EIE Instruments Pvt. Ltd., Ahmedabad, India) was used for the determination of the melting point of idelalisib and a digital pH meter (Eutech Instruments, model GC7252101B) was used to adjust the pH of the buffer solution.

Standard stock solution for spectrofluorimetry (1000.0 μg/mL) was prepared daily by dissolving 10.0 mg of idelalisib in 10 mL of the solvent (0.1 N HCl or absolute ethanol), which was diluted 1 in 10 to obtain a stock solution (100 μg/mL). The working standard solutions, ranging from 0.1 to 100.0 μg/mL of idelalisib were prepared by serial dilutions of the stock solution with the appropriate solvent and the test tubes were kept stoppered to avoid evaporation of the solvent.

The excitation and emission spectra for the working standard solutions of idelalisib (ranging from 0.1 to 100.0 μg/mL) were recorded over the wavelength range of 200–400 and 400–800 nm, respectively. The maximum absorbance of the drug (λ_{max}) was observed at 270 and 330 nm in ethanol and 0.1 N HCl, re-

spectively, and these were selected as the respective excitation wavelengths for the spectrofluorimetric assay. Fluorescence intensities of solutions in absolute ethanol were recorded taking, respectively, 330 and 650 nm as the excitation and emission wavelengths against the reagent blank (method 1). The fluorescence intensities of solutions prepared in 0.1 N HCl were recorded, respectively, taking 270 and 350 nm as the excitation and emission wavelengths against the reagent blank (method 2). The optimized method was validated with respect to various parameters outlined in ICH guideline Q2 (R1).

The drug concentration selected for stress studies was 1.0 mg/mL. Hydrolytic studies were carried out under acidic and basic conditions by refluxing the drug in 0.1 N HCl and 0.1 N NaOH, respectively, at 80°C for 8 h. Photodegradation studies were carried out at 40°C by exposing a thin layer of the solid drug in a Petri dish, and also the drug solutions were prepared in 0.01 N HCl, to a total dose of 1.2 million lux h of fluorescent and 200 Wh/m^2 of UV-A illumination in a photostability chamber, whereby they were placed ~9 inches from light sources for 10 days.

Oxidative studies were carried out at room temperature in 30% hydrogen peroxide (H_2O_2) for half an hour. Thermal degradation was carried out by exposing the drug (200 mg) in a Petri dish, sealed with aluminium foil (to avoid photo-degradation), to a temperature of 60°C for 21 days. Subsequently, the Petri dish was removed, cooled to room temperature and its contents were dissolved in acetonitrile (diluent).

The stability-indicating potential of the developed methods was evaluated by fortifying a preneutralized, equal-volume mixture of stress-degraded solutions of idelalisib prepared under conditions of acidic/alkaline hydrolysis, acid photolysis, and oxidative stress. The original drug concentration in all the stressed solutions was the same, i.e., 1.0 μg/mL for method 1 (in absolute ethanol) and 10.0 μg/mL for method 2 (in 0.1 N HCl), but due to varying degradation profiles under different conditions, the fluorescence intensities of the solutions were correspondingly reduced.

Twenty tablets of idelalisib (Zydelig™, Gilead sciences) with a label claim of 100 mg per tablet were weighed, crushed, and powdered. A powder weight equivalent to 10 mg of idelalisib was suspended in ethanol/0.1 N HCl, sonicated for 5 min and filtered. The volume was made up to 100 mL (final drug solution 100 μg/mL). The solutions were suitably diluted and these dilutions were analyzed thrice for the drug content.

Results and discussion. Idelalisib is a quinazolinone derivative further substituted with a phenyl group creating an extended conjugated system of unsaturation in the molecule in a planar orientation. The partition coefficient (log*P*) for idelalisib is 2.0 and the pK_a is 1.6, 3.4, and 9.8. The drug demonstrates a pH-dependent solubility in an aqueous medium ranging from <0.1 mg/mL at pH 5–7 to over 1 mg/mL at pH 2 under ambient conditions [15]. The drug is not soluble at higher pH ranges. Systematic analysis of fluorescence characteristics of the drug was carried out in various solvents and buffers, revealing that the drug possesses a very good native fluorescence in the acidic medium as well as in ethanol without the requirement of any type of fluorimetric enhancers. Based on preliminary absorption/fluorescence studies and solubility characteristics of the drug, which was more soluble in strongly acidic buffers with pH ranges close to 2, 0.1 N HCl and absolute ethanol were selected for fluorimetric analysis of idelalisib. The excitation and emission spectra for the working standard solutions of idelalisib ranging from 0.01 to 100.0 μg/mL were recorded over the ranges of 210–400 and 400–800 nm, and respective concentration ranges demonstrating linearity were taken for calibration plots. In absolute ethanol, the excitation and emission wavelengths for spectrofluorimetric analysis were selected as 330 and 595 nm, respectively. The UV absorption and fluorescence characteristics of the drug were significantly changed in hydrochloric acid and the fluorescence intensity in 0.1 N HCl was determined taking 270 and 350 nm as the excitation and emission wavelengths respectively against the solvent blank. Figure 1 shows the emission/excitation scans in absolute ethanol and 0.1 N HCl, respectively. The drug was also very soluble in acetate buffer (pH 2.8) with similar fluorescence characteristics and good fluorescence intensity (ranging from 60 to 210 FU, but linearity and reproducibility was found to be much lower in comparison with HCl. In methanol, also same good fluorescence intensity was seen ranging from 40–150 FU; however, validation parameters (particularly, linearity and range) were found to be better in ethanol.

The developed methods were validated with respect to linearity, range, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) and robustness. The various validation parameters are summarized in Tables 1 and 2. Stability, indicating the nature of the assays, was assessed by fortifying a mixture of degraded solutions with three known concentrations of the drug. The recovery of the added drug was then determined.

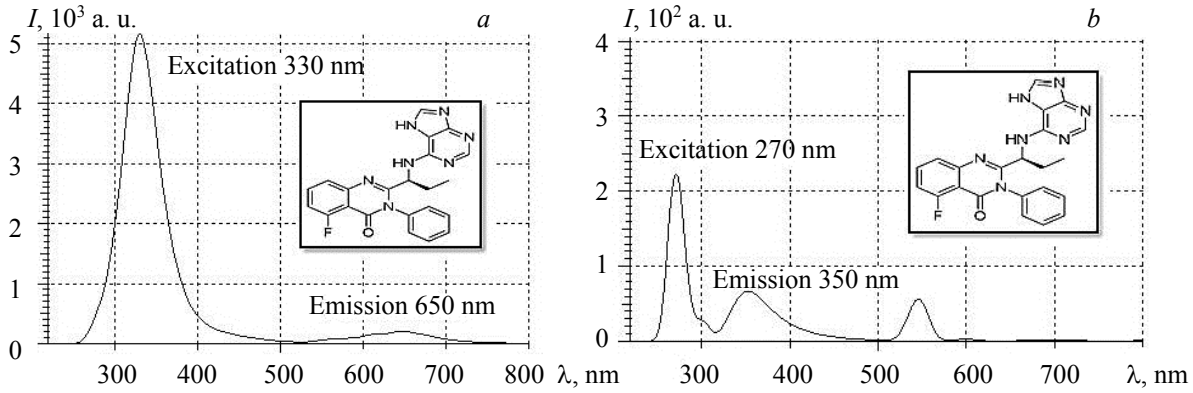

Fig. 1. Excitation and emission scans for spectrofluorimetric analysis of idelalisib by method 1 (a) and method 2 (b).

TABLE 1. Validation Parameters for Spectrofluorimetric Analysis of Idelalisib by Method 1

Accuracy	Concentration (μg/mL) ± S.D.; %RSD[#]			
	Conc. of drug taken, μg/mL	Concentration of standard added, μg/mL[*]	Calculated[**]	%Recovery
	0.5	0.5 (50%)	0.92± 0.01; 1.08	92.12
	0.5	1.0 (100%)	1.46± 0.02; 1.37	97.66
	0.5	1.5 (150%)	1.98± 0.01; 0.51	99.02
Precision		Calculated concentration (μg/mL) ± S.D.; %RSD		
	Conc. taken, μg/mL	Intra-day (n = 6)	Inter-day (n = 3)	
	0.5	0.46± 0.01; 2.17	0.46±0.01; 2.17	
	1.0	0.92 ± 0.01; 1.08	0.94±0.02; 2.12	
	2.0	1.96 ± 0.01; 0.51	1.95± 0.02; 1.03	
Linearity		Range, μg/mL	Slope	Intercept	r^2
		0.1–2.0	57.96	69.69	0.9993
LOD & LOQ		0.015 and 0.045 μg/mL			
Recovery (± SD); %RSD in tablet samples (label claim 100 mg/tablet)				98.02 ± 1.69 mg; 1.33% 98.02 ± 0.029 mg; 2.98%	

[*]Diluted degraded drug solution (0.5 μg/mL) mixed with equal volumes of the standard drug solutions with concentrations 1.0, 1.5, and 2.0 μg/mL.
[**]Calculated as mean of three measurements (n = 3).
[#]Calculated as 100 SD/mean.

A strictly linear relation was observed between the fluorescence intensity and concentration of idelalisib (in absolute ethanol; method 1) in the concentration range of 0.1–2.0 μg/mL. The corresponding calibration curve was described by the equation $y = 57.959x + 69.685$ (n = 6, $r^2 = 0.9993$) (Fig. 2a). The linearity range for the fluorimetric method performed in 0.1 N HCl (method 2) was 0.1–20.0 μg/mL and the corresponding calibration curve was described by the equation $y = 19.547x + 39.879$ (n = 7, $r^2 = 0.9998$) (Fig. 2b).

The LOD and LOQ were calculated using the formulae (3.3 σ/s) and (10 σ/s), respectively, where σ is the standard deviation of the response (calculated from the standard deviation of intercept) and s is the slope of the calibration curve. The slopes and intercepts of calibration plots for three sets of fluorescence intensities (from linearity studies) were calculated and taken for the calculation of the LOD and LOQ values. The LOD and LOQ values in method 1 were found to be 0.0150 and 0.045 μg/mL, respectively, whereas for method 2, these values were found to be 0.1615 and 0.4890 μg/mL, respectively (Tables 1 and 2). Furthermore, solutions of the drug having concentrations corresponding to LOD and LOQ values were prepared and analyzed six times (n = 6).

TABLE 2. Validation Parameters for Spectrofluorimetric Analysis of Idelalisib by Method 2

Accuracy	Calculated concentration (μg/mL) ± S.D.; %RSD[#]				
	Concentration of drug taken, μg/mL	Concentration of standard added, μg/mL[*]		Calculated[**]	%Recovery
	10.0	5.0 (50%)		14.83±0.30; 2.02.	98.93
	10.0	10.0 (100%)		19.88± 0.23; 1.16	99.44
	10.0	15.0 (150%)		23.26±1.81; 0.01	93.05
Precision	Calculated concentration (μg/mL) ± S.D.; %RSD				
	Conc. taken, μg/mL	Intra-day (n = 6)		Inter-day (n = 3)	
	5.0	4.84 ± 0.07; 1.44		4.71 ± 0.12; 2.54	
	10.0	9.86 ± 0.09; 0.91		9.79 ± 0.17; 1.73	
	15.0	14.98 ± 0.11; 0.73		14.91 ± 0.10; 0.67	
Linearity		Range, μg/mL	Slope	Intercept	r^2
		0.1–20.0	19.547	39.879	0.9998
LOD & LOQ		0.1615 and 0.489 μg/mL			
Recovery (± SD); %RSD) in tablet samples (label claim 100 mg/tablet)				98.02 ± 1.69 mg; 1.72% 98.30 ± 0.60 mg; 0.61%	

[*]Diluted degraded drug solution (10.0 μg/mL) mixed with equal volumes of the standard drug solutions with concentrations 20.0, 30.0, and 40.0 μg/mL.
[**]Calculated as mean of three measurements (n = 3).
[#] Calculated as100 SD/mean.

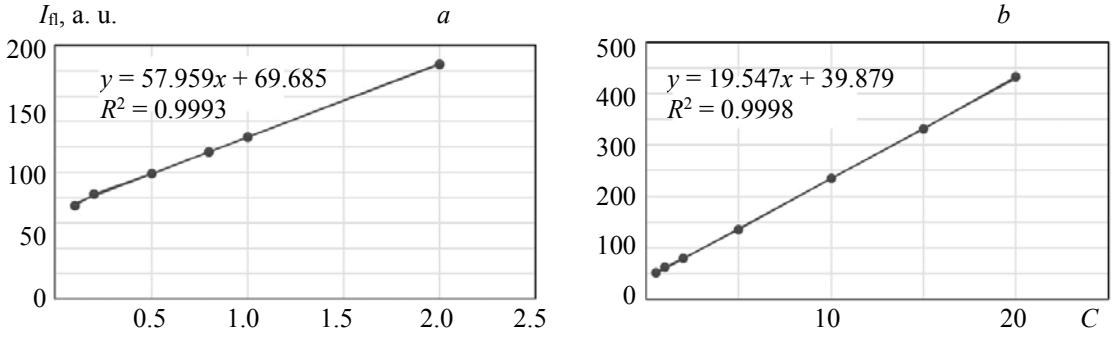

Fig. 2. Calibration plot for spectrofluorimetric analysis of idelalisib by methods (a) 1 and (b) 2.

Different concentration levels of the drug for analysis were prepared from independent stock solutions. Assessment of the accuracy of the developed methods was done by fortifying excess drug (50, 100, and 150%) to pre-analyzed drug solution samples (0.5 μg/mL for method 1 and 10 μg/mL for method 2; Tables 1 and 2). Accuracy was determined as the mean % recovery of the fortified drug concentration. Good recoveries were obtained in both of the methods, i.e., 92.12–99.02% (method 1) and 93.05–99.44% (method 2).

Both methods were found to be sufficiently precise with low %RSD values for the intra-day and inter-day precision (below 0.99 and 1.64% in method 1; below 0.52% and 0.94% in method 2; Tables 1 and 2). This showed that the methods were sufficiently precise for determining the drug concentrations.

The methods were found to be robust as no significant changes in fluorescence intensity were observed when carrying out deliberate changes in the method variables including the excitation wavelength, emission wavelength, and analyst performing the study. The %RSD values in all cases were found to be less than 1.31% (absolute ethanol) and 1.71% (hydrochloric acid).

No significant change in fluorescence intensity was observed when carrying out deliberate changes in the method variables, including the excitation wavelength, emission wavelength and the analyst performing the study. Hence, the method was found to be robust with the %RSD in all cases being less than 2.6%; the

results from the robustness studies are shown in Table 3. The responses with fluorescence measurements were found to be stable for at least 8 h at room temperature, indicating the stability of the final sample solutions for at least 8 h.

TABLE 3. Robustness of the Proposed Methods

Parameter	Change	Fluorescence intensity			Mean	SD	%RSD
Method 1							
Optimized conditions	NA	129.2	128.7	127.6	128.3	0.82	0.64
Excitation λ_{max} = 330 nm	335	110.9	112.6	109.7	111.1	1.46	1.31
Emission λ_{max} = 650 nm	655	116.8	115.3	114.7	115.6	1.08	0.94
Analyst I	Analyst II	125.4	126.7	123.6	127.3	1.55	1.22
Method 2							
Optimized conditions (5 μg/mL)	NA	134.9	135.1	136.3	135.4	0.75	0.55
Excitation λ_{max} = 270 nm	275	105.8	108.6	109.3	107.9	1.85	1.71
Emission λ_{max} = 350 nm	355	118.6	116.3	115.4	116.7	1.65	1.45
Analyst I	Analyst II	133.8	135.3	136.3	134.0	1.21	0.90

Maximum degradation of idelalisib was noted under alkaline conditions (percent recovery 29.8 and 32.11% in methods 1 and 2, respectively), followed by acidic conditions (recovery 47.77 and 44.8% in methods 1 and 2), oxidative conditions (47.64 and 47.9% in methods 1 and 2), photolytic conditions (63.6 and 64.3% in methods 1 and 2), and under thermal conditions (recovery of 97.5% and 96.2% in methods 1 and 2, respectively), in this order.

The final drug solution (100 μg/mL) obtained by sonication of the tablet powder (Zydelig™, Gilead sciences; label claim 100 mg) in absolute ethanol/0.1 N HCl was suitably diluted and the fluorescence intensity of the resulting dilutions was noted. Table 4 shows the results of the assay by the proposed methods. Good recovery was obtained with both methods, i.e., 98.02% (method 1) and 98.30% (method 2), thereby showing a close agreement between the results obtained by the proposed methods and the label claim (100 mg per tablet).

TABLE 4. Recovery Studies with Marketed Drug Formulation Zydelig™
(Gilead sciences; label claim 100 mg)

Method	Mean recovery (mg) ± S.D; %RSD	%Recovery ± S.D; %RSD
1	98.02 ± 1.69; 1.72	98.02 ± 1.69; 1.72
2	98.30 ± 0.60; 0.61	98.30 ± 0.60; 0.61

Conclusions. Sensitive stability-indicating spectrofluorimetric methods have been proposed for the determination of idelalisib in bulk as well as in its marketed formulation (tablets) utilizing the native fluorescence of the drug. This is the first-ever report on the spectrofluorimetric analysis of this drug. The fluorescence characteristics of the drug were found to differ significantly in a neutral (ethanol) and acidic (hydrochloric acid) medium. Computed validation parameters suggest the methods to be sufficiently precise, accurate, reproducible, and robust with solution stability for up to 8 h. Good recoveries of the drug from degraded drug solutions indicate the stability-indicating potential of the methods. The proposed methods have been successfully used to quantify the drug in its marketed tablet formulation with good recoveries, suggesting that the method is well suited for routine drug analysis without any interference from the formulation excipients.

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