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Simultaneous estimation of gliclazide and lacidipine using spectrophotometric methods and its application in nanostructured lipid carrier (NLC) development

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ABSTRACT

Simple, accurate, and precise methods for simultaneous estimation of Gliclazide (G_{ld}) and Lacidipine (L_{dpn}) in combined-dosage form have been described. Techniques used employ 1st order derivative and simultaneous equationmethod for the simultaneous estimation of the drugs in combination dosage form. Linearity was observed in the concentration range of 2-20 µg/mL for $G_{ld}$and 4-18 µg/mL for L_{dpn}. The accuracy of methods was assessed by recovery studies and was found to be within a range of 98-102% for both G_{ld} and L_{dpn}. The developed methods were validated with respect to linearity, accuracy (recovery), and precision. The results were validated statistically as per ICH Q2 R1 guideline and were found to be satisfactory. The proposed methods were successfully applied for the determination of G_{ld} and L_{dpn} in the NLC formulation.

Keywords: Gliclazide, Lacidipine, 1st order derivative, Simultaneous estimation, UV spectrophotometry.

INTRODUCTION

Gliclazide (G_{ld}) is chemically N-(hexahydrocyclopenta[c]pyrrol-2(1H)-ylcarbamoyl)-4-methylbenzene sulfonamide[Fig. 1 (A)]and is widely used as an oral hypoglycemic drug appertaining to the second generation class of sulphonylureas. Its indication is mainly for patients suffering from diabetes mellitus type 2 [1]. It is frequently ubiquitous of diabetic patients to be at a high peril of polypharmacy and thus complex medication regimens. Much prevalent concurrent diseases such as hypertension leading to cardio-vascular morbidity in diabetes affected patients are treated by radical and comprehensive control of BP using low-dose diuretics, calcium channel antagonists, β-blockers or angiotensin converting enzyme inhibitors, as a first-line treatment [2]. As an outcome of extensive trials conducted to study various drugs pertaining to different categories, to obtain data for lowest mortality rate in about 5,000 hypertensive diabetics, calcium channel blocker (CCB) based therapy was found to give optimum results in the data collected. Members of only the dihydropyridines class of CCB (nifedipine, amlodipin and lacidipine) are used in hypertensive-diabetic patients [3]. Although there have been reports where ARB or ACE-I is to be advised as a first line treatment for hypertensive-diabetic patients, CCB is more favorable in reducing HBPV. Investigative reports have shown decidedly lower coefficient variation of morning systolic BP in CCB treated patients as compared to angiotensin II receptor blockers and/or ACE-I treated groups [4].

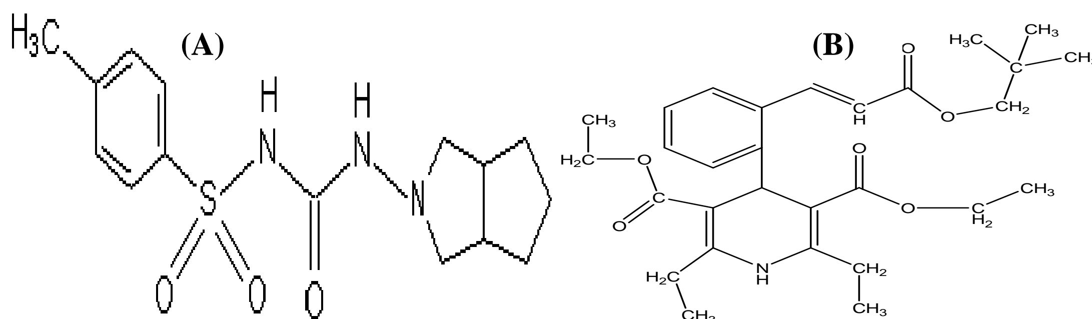

Fig. 1: Structure of G_{ld}(A) and L_{dpn}(B)

Lacidipine (L_{dpn}) is chemically diethyl 2,6-dimethyl-4-(2-{(1E)-3-[(2-methyl-2-propanyl)oxy]-3-oxo-1-propen-1-yl}phenyl)-1,4-dihydro-3,5 pyridinedicarboxylate [Fig. 1 (B)], a drug belonging to the dihydropyridine category of CCB. In adjunction to its antihypertensive activity, L_{dpn} also shows anti-atherosclerotic and antioxidant actions, beneficial to patients suffering from hypertension and related disorders [5]. L_{dpn} when studied in diabetic hypertensive patients was found to reduce mean 24-hour blood pressure, blood pressure variability and simultaneous amelioration of baroreflex sensitivity [6].Additionally, according to another study performed on patients suffering from type 2 diabetes advocated L_{dpn} to not possess any negative effects on metabolic parameters such as total cholesterol, triglycerides, high-density lipoproteins and blood glucose [7-8]. In addition to the aforementioned, G_{ld} level in serum and pharmacokinetic parameters such as like AUC, AUMC, $T_{1/2}$, Clearance, V_{dss}, V_{darea}, Cmax and Tmax were positively affected when administered with single and multiple dose therapies of L_{dpn}. Metabolism of both drugs happens extensively by liver enzymes. Both L_{dpn} and G_{ld} get extensively metabolized by CYP450 3A4 and P450 CYP2C9 respectively to give pharmacologically inactive metabolites [9]. This results in negligible interaction amongst the drugs at metabolic level. Sulphonylureas target ATP sensitive K^+ channels and act in patients of type 2 diabetes by stimulating pro-insulin secretion owing to closing of K^+ATP channels in pancreatic cells. Also, both drugs L_{dpn} and G_{ld}have comparable mean half-life, i.e. L_{dpn} ranging between 13.2 to 18.7 h and G_{ld} ranging between 12 to 20 h which allow the two to be effectively administered using combination treatment. [10-12].

Henceforth, combination of G_{ld} and L_{dpn} is a therapeutically viable, safe and competent option for treatment of hypertensive-diabetic patients. Although both show common shortcomings of poor solubility and extensive first pass hepatic metabolism, hampering their oral bioavailability and leading to significant inter-individual variations [13]. A transdermal formulation can be a possible solution for the successful delivery of the combination. There are many reported methods to estimate either G_{ld} [14] or L_{dpn} [15] alone, but to the best of our knowledge, simultaneous determination of G_{ld} and L_{dpn} has not yet been reported elsewhere.

This paper aims to describe the development and validation of the UV Spectrophotometric method for the simultaneous determination of G_{ld} and L_{dpn}.

MATERIALS AND METHODS

Material:
G_{ld} and L_{dpn} was obtained from Manus Aktteva, Gujarat, India. HPLC grade Methanol was procured from Merck (Mumbai, India). Soya phosphatidylcholine was purchased from Sigma Aldrich (St. Louis, USA). Virgin coconut oil was obtained from Harin Biotech International Pvt. Ltd. (Bangalore, India). Freshly collected Milli-Q water filtered through a 0.22mm membrane filter was used in all set of experiments.Double beam UV/Vis spectrophotometer, Shimadzu UV 1800 with 1 cm quartz cells was used.

Methods:
Preparation of NLCs:
G_{ld}(40 mg), L_{dpn}(2 mg) and 1:3:2% w/w ratio of soya lecithin:stearic acid:virgin coconut oil was dissolved in 50 ml of chloroform. Chloroform was evaporated under vacuum (–760 mmHg)at 55°C, using rotary evaporator (Heidolph, Schwabach, Germany) to remove chloroform (lipid phase). Aqueous phase (200 mL) containing Tween 20 (2% w/v) was heated on a water bath upto80°C and mixed to the lipid phase under homogenization at 8000 rpm (Kinametica, Polytron® PT-MR3100D, New York, USA) for 3 minto obtain a pre-emulsion. Pre-emulsion was finally homogenized at 80°C through the high pressure homogenizer (GEA Niro Soavi, model PANDA plus, Italy) for 3 cycles at 650 bars. The prepared hot o/w nanoemulsion was then allowed to cool at room temperature [16].

Preparation of standard drug solutions:
Standard stock solutions containing Gliclazide (G_{ld}) and Lacidipine (L_{dpn}) were prepared by dissolving an accurately weighed quantity of G_{ld}(10 mg) and L_{dpn}(10 mg)separately in 2 ml volumetric flask with methanol, selected as a common solvent. It was then sonicated for 10 min and further diluted upto 10mL with methanol to get a stock solutions of1000 µg/mL concentration.

Working standard stocksolution:
From previously prepared stock solution (1000 µg/mL), 1 mL of the solution was pipetted out and diluted with methanol in order to obtain a working standard stock solution of 100 µg/mL.

Selection of analytical wavelength:
Stock solutions of Gliclazide (G_{ld}) and Lacidipine (L_{dpn}) were diluted to obtain final concentration of 2-20 µg/mL for G_{ld} and 4-18 µg/mL for L_{dpn},separately. Each of the working standard solutions wasscanned between 400 and 200 nm at a medium scanningspeed. It showed wavelength maxima at 229 nm for G_{ld} and 240 nm for L_{dpn}[Fig. 2].

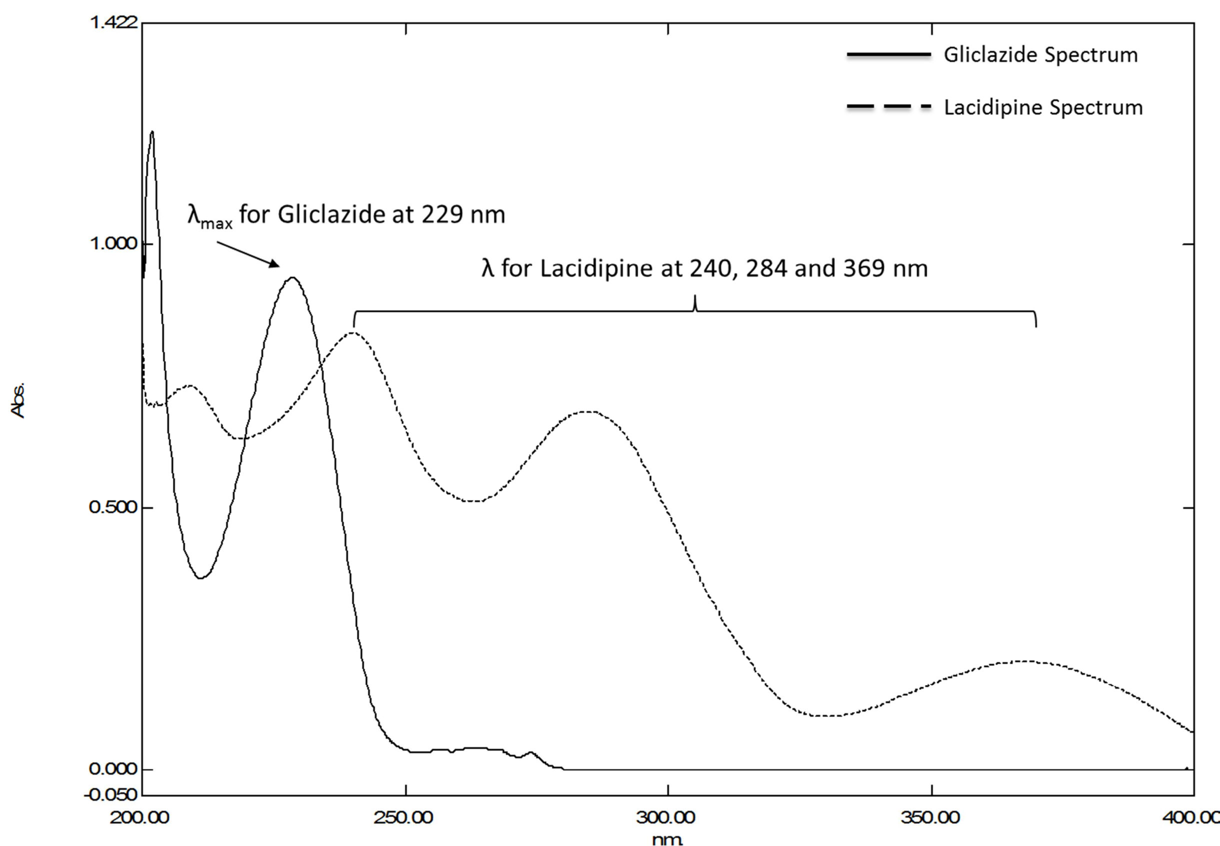

Fig.2: Overlay spectra of G_{ld} and L_{dpn}

Method I [17]:
All the zero order spectra were then transformedto their respective first order derivative spectra using the UVProbev2.35 software and zero crossing points for G_{ld} and L_{dpn}were found to be at 220 nm and 300 nm, respectively. Responses of each of the abovesolutions were measured for G_{ld} and L_{dpn}at 220 nm and 300 nm, respectively. The calibration curves wereconstructed[Fig. 3].

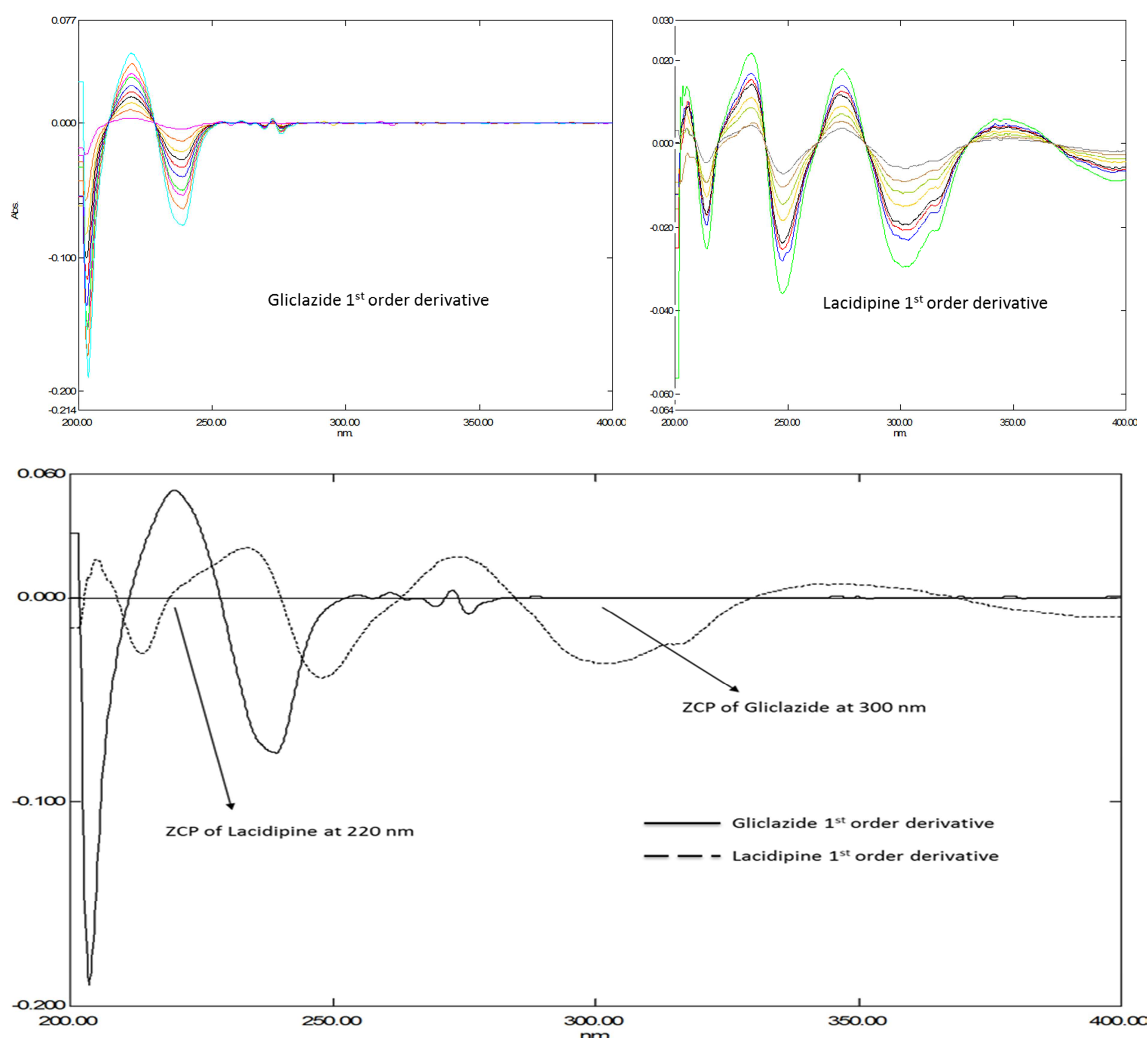

Fig. 3: 1st order derivative spectra of G_{ld} and L_{dpn}

Method II [17]:
The absorbance of G_{ld} and L_{dpn}solutions was measured at 229 and 240 nm, and the calibration curves were plotted at these wavelengths. The absorptivity coefficients of these two drugs were determined using the calibration curve equations. The concentration of G_{ld} and L_{dpn} in the sample solution was determined by solving the respective simultaneous equations generated by using absorptivity coefficients and absorbance values of G_{ld} and L_{dpn}at these wavelengths.

Two simultaneous equations as givenbelow were formed using absorptivity values, A(1%, 1cm)

At λ^1 $A^1 = ax^1Cx + ay^1Cy$ (1)
At λ^2 $A^2 = ax^2Cx + ay^2Cy$ (2)

Where,
ax^1 =A (1%, 1cm) of G_{ld} at 229 nm (4932.8)

ax^2 =A (1%, 1cm) of G_{ld} at 240 nm (2689.3)

ay^1=A (1%, 1cm) of L_{dpn} at 229 nm (3398.0)

ay^2 = A (1%, 1cm) of L_{dpn} at 240 nm (4549.9)

$Cx = (A^2ay^1 - A^1ay^2) / (ax^2ay^1 - ax^1ay^2)$ (3)

$$Cy = (A^1ax^2 - A^2ax^1) / (ax^2ay^1 - ax^1ay^2) \quad (4)$$

Validation of the Methods [18]:
Newly developed methods(I and II)were validated for specificity, linearity, accuracy, precision, limits of quantitation, and limits of detection according to the ICH guideline Q2 (R1).

Linearity and Range: For the Ist orderderivative method and simultaneous equation method, the linearity response was determined byanalyzing independent calibration curves in the range of 2-20μg/mL for $G_{ld}$and 4-18μg/mL for L_{dpn}. Correlation coefficient and regressionline equations for G_{ld} and L_{dpn}were calculated for both themethods (Table 1).

Precision:
Repeatability:The repeatability was checked by scanning and measurement of the responses of solutions of $G_{ld}$and L_{dpn} (10 μg/mL, each) without changing the parameters of the method I and II. The procedure was repeated six times and % RSD was calculated (Table 1).

Intermediate Precision:The intraday and interday precisions of the both the methods were determined by analyzing corresponding responses on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of $G_{ld}$and L_{dpn}(6, 10, and 14μg/mL) ((Table 3 and 4).

Table 1: Various parameter obtained from Method I and II

Validation parameters	Method I		Method II			
	G_{ld}	L_{dpn}	G_{ld}		L_{dpn}	
Linearity and range (μg/mL)	2-20	4-18	2-20		4-18	
Wavelength (nm)	220	300	229	240	229	240
Correlation coefficient (r^2)	0.9913	0.9928	0.9997	0.9997	0.998	0.9935
Slope	0.0025	-0.0016	0.0407	0.0214	0.0344	0.0531
Intercept	-0.0006	0.0005	0.0593	0.0384	-0.0043	-0.0676
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	-	-	159526.75	86971.96	154792.49	207266.14
Sandell's Sensitivity ($\mu g/cm^2/0.001$)	-	-	0.0206	0.0380	0.0294	0.0221

Table 2: Repeatability study data for mixture of G_{ld} and L_{dpn} (n=6)

Drug	Concentration (μg/mL)	Method I		Method II	
		% Calculated	% RSD	% Calculated	% RSD
G_{ld}	10	99.68 ± 0.42	0.42	98.76 ± 0.89	0.89
L_{dpn}	10	99.08 ± 0.28	0.28	97.89 ± 0.74	0.74

Table 3: Intraday precisiondata for mixture of G_{ld} and L_{dpn} (n=6)

Drug	Concentration (μg/mL)	Method I		Method II	
		% Calculated	% RSD	% Calculated	% RSD
G_{ld}	6	99.98 ± 1.12	1.12	97.86 ± 1.89	0.89
	10	100.24 ± 0.82	0.82	99.63 ± 0.45	0.45
	14	101.43 ± 1.52	1.52	97.80 ± 1.24	1.24
L_{dpn}	6	99.08 ± 0.28	0.28	100.85 ± 1.84	1.84
	10	100.34 ± 0.78	0.78	101.29 ± 1.04	1.04
	14	100.44 ± 0.18	0.18	99.09 ± 0.42	0.42

Table 4:Interday precisiondata for mixture of G_{ld} and L_{dpn} (n=6)

Drug	Concentration (μg/mL)	Method I		Method II	
		% Calculated	% RSD	% Calculated	% RSD
G_{ld}	6	100.09 ± 0.32	0.32	99.83 ± 1.09	1.09
	10	100.44 ± 0.60	0.60	100.78 ± 1.05	1.05
	14	100.73 ± 0.56	0.56	100.98 ± 0.24	0.24
L_{dpn}	6	100.88 ± 0.48	0.48	100.20 ± 0.24	0.24
	10	99.84 ± 0.27	0.27	100.54 ± 0.14	0.14
	14	100. 67 ± 0.91	0.67	101.79 ± 0.32	0.32

Accuracy:It was carried out to determine the suitability and reliability of the proposed methods. Accuracy was determined by calculating the % recovery of G_{ld} and L_{dpn} from the synthetic mixture by the standard addition method in which known amounts of standards samples of G_{ld} and L_{dpn} were added to the preanalysed samples. The procedure was repeated 5 more times and the recovered amounts of G_{ld} and L_{dpn} were calculated at each level and % recovery was reported as % recovery = $((C_{total} - C_{assay})/C_{added})\times100$, where C_{total} is the total drug concentration found after standard addition, C_{assay} is the drug concentration in the formulation mixture, and C_{added} is the concentration of standard added (Table 5).

Table 5: Recovery study data for G_{ld} and $L_{dpn}$by Method I and Method II(n=6)

Drug	Pre-analyzed conc.	Drug added (µg/mL)	Method I		Method II	
			% Recovery	% RSD	% Recovery	% RSD
G_{ld}	4 µg/mL	-	-	0.88	-	0.46
		2	100.39 ± 0.42	0.42	101.43 ± 1.04	1.04
		4	101.67 ± 0.22	0.22	100.18 ± 1.56	1.56
		6	101.54 ± 0.47	0.47	99.98 ± 0.46	0.46
L_{dpn}	4 µg/mL	-	-	0.69	-	0.33
		4	101.74 ± 0.67	0.67	101.89 ± 1.54	1.54
		8	100.23 ± 0.42	0.42	100.91 ± 0.19	0.19
		12	100. 54 ± 1.08	1.08	101.65 ± 0.46	0.46

Analysis of NLC formulation:
The EE % of prepared NLCs was determined through ultrafiltration technique. For this purpose 5 mL of NLC dispersion was centrifuged (Mikro-220R, Hettich Zentrifugen, Tuttlingen, Germany) at 12,000 rpm for 20minat 5°C using centrifugal filter tubes (Centrisart, Sartorius AG, Goettingen, Germany) having a filter membrane of 20 kDa molecular weight cutoff. The amount of G_{ld} and L_{dpn} in the filtered aqueous phase was measured usingfirst order derivative (Method I) and vierodt's (Method II). Results of analysis are shown in Table 6.

Table 6: Entrapment Efficiency (%) of G_{ld} and $L_{dpn}$by Method I and II(n=6) in NLCs

Drug	Entrapment Efficiency (%)	
	Method I	Method II
G_{ld}	68.32 ± 2.94	70.46 ± 8.21
L_{dpn}	79.09 ± 3.43	75.33 ± 6.34

RESULTS AND DISCUSSION

The methods discussed in the present work provides a useful, precise and accurateway for simultaneous analysis of G_{ld} and L_{dpn} with help of vierodt's and I^{st} order derivative method. Absorbance maxima of G_{ld} at 229 nm and L_{dpn} at 240 nm were selected for the analysis. Regression analysis showed a linearity over the concentration range of 2-20 µg/mL for G_{ld} and 4-18 µg/mL for L_{dpn} with respective correlation coefficients of 0.9913 and 0.9928, respectively in case of 1st order derivative method. % RSD for repeatability (n=6), interdayand intraday(n=6) precision was found to be less than 2% signifying the precision of method.Accuracy of proposed methods was establishedwith recovery studies. The percentage recovery for G_{ld} and $L_{dpn}$was found well within the range of 98 % and 102%. Values of standard deviation and coefficient of variation was satisfactorily low indicating the accuracy of both the methods.The % RSD value for both the drug was found to be less than 2%.The percent recovery value (Table 2), higher than 100%, indicates the accuracy of the method. The estimationof G_{ld} and L_{dpn}in NLC formulationwas foundto be 68.32 ± 2.94 and 79.09 ± 3.43 with method I and 70.46 ± 8.21 and 75.33 ± 6.34 with method II for G_{ld} and L_{dpn}, respectively.

In this study the simultaneous estimation of G_{ld} and $L_{dpn}$was carried out by derivative and simultaneous equation methods satisfactorily.

CONCLUSION

Based on these results, it is concluded that the UV-spectrophotometric technique developedis accurate, sensitive,precise, reproducibleand economical. Itcan become an effective analytical tool for routine quality control of G_{ld} and L_{dpn}in combination. Also, the method developed is specific while estimating NLC formulations and showed no interference of the excipients used.

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