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NOVEL SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS QUANTITATION OF DAPAGLIFLOZIN PROPANEDIOL MONOHYDRATE, LINAGLIPTIN AND METFORMIN HYDROCHLORIDE IN SYNTHETIC MIXTURE

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ABSTRACT

Dapagliflozin propanediol monohydrate (DAPA), Linagliptin (LINA) and Metformin Hydrochloride (METF) is a fixed-dose combination administered to patients with diabetes mellitus to improve glycemic control. A novel spectroscopic technique; Double Divisor Ratio Spectra Derivative method (DDRSD) was developed and validated for the concurrent analysis of DAPA, LINA and METF without prior separation. This approach involves dividing the absorbance spectra of the ternary blend by the summation of the absorbance spectra of 2 out of 3 components, resulting in the generation of ratio spectra, which is subsequently subjected to the first derivative. The analyte was estimated at minima for DAPA (282 nm) and LINA (316.74 nm) and at maxima for METF (236 nm). The proposed approach was validated as per ICH guideline. Linearity was demonstrated over the range 0.5 - 25 µg/ml, 0.2 - 14 µg/ml and 5 - 50 µg/ml for DAPA, LINA and METF with correlation coefficients of 0.9996, 0.9991 and 0.9994 correspondingly. The percent recoveries were in the range of 98.6 – 101 % for DAPA, 99.3 – 101.3 % for LINA and 100.3 – 101 % for METF. The percent RSD for precision was less than 2. A novel simple, precise and accurate UV spectroscopic method was established and validated for the simultaneous analysis of DAPA, LINA and METF in a synthetic mixture. This approach can be

successfully employed for concurrent QC analysis of DAPA, LINA and METF in synthetic mixture and dosage form.

Keywords: Dapagliflozin propanediol monohydrate, Linagliptin, Metformin Hydrochloride, Double divisor ratio spectra derivative

INTRODUCTION

Currently, diabetes is the most prevalent disease globally. The results of recent research indicate that Type 2 diabetes (T2D) is becoming more common in society. Glycosylated hemoglobin (HbA1C) is continuously elevated in T2D, a disease that progresses over time and is linked to a higher risk of microvascular and macrovascular complications as well as a significantly shorter life expectancy. T2D is caused by three main factors: problems with how the body secretes insulin, too much glucose being produced by the liver, and resistance to the effects of insulin in tissues like muscles, liver and fat cells. In the treatment of this disease, oral insulin formulations play a crucial role, including, biguanides, or secretagogues (sulphonylureas/glinides) and Dipeptidyl peptidase-4 respectively **[1, 2]**.

DAPA, represented by **Fig. 1(A)** is categorized as a sodium glucose co-transporter 2 (SGLT2) inhibitor is chiefly responsible for the kidneys' reabsorption of glucose. DAPA serves as an adjunctive therapy for T2D in conjunction with controlled dietary habits and regular physical activities. It is frequently prescribed alongside other medications.

LINA, denoted as **Figure 1(B)**, functions as a competitive and reversible inhibitor of Dipeptidyl peptidase 4 (DPP-4). This inhibition results in delayed disruption of Glucagon-like peptide-1 (GLP-1) and glucose-dependent Gastric inhibitory polypeptide (GIP). LINA is employed to treat type 2 diabetes.

METF, depicted as **Figure 1(C)**, it is commonly prescribed for type 2 diabetes mellitus management and frequently employed in obesity management. Despite its capacity to stimulate insulin secretion, METF exerts its anti-diabetic effects even in the absence of substantial insulin levels **[3-9]**.

DAPA (10 mg), LINA (5 mg) and METF (500 mg) approved as FDC tablet that enhance the heme glucose level in patients with T2D **[10].** The research revealed that UV spectroscopic and chromatography technique has been published for the determination of DAPA **[11-24]**, LINA **[25-36]** and METF **[37-51]** in single drug and with other drugs.

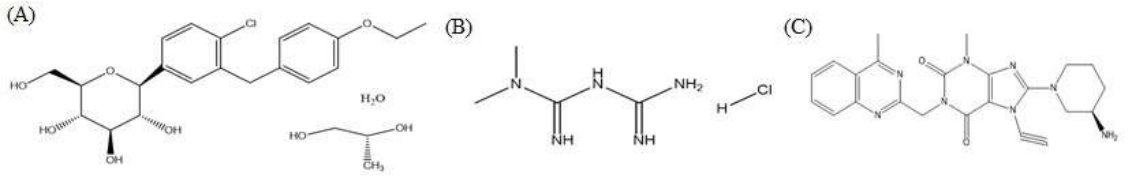

Figure 1: Structure of (A) DAPA, (B) LINA and (C) METF

No spectroscopic technique has been reported for the concurrent determination of DAPA, LINA and METF in synthetic mixture or formulation. In this combination strongly overlapped spectra were obtained so, direct UV spectroscopic estimation is not appropriate. By directly measuring the amplitudes at peaks or minima, the ratio spectra have an advantage over the zero-crossing derivatives in terms of enhanced sensitivity and selectivity prior to that of the zero-crossing points. Furthermore, a number of advanced instrumental techniques based on separation, such as capillary electrophoresis and HPLC, enable the separation and assessment of sample content; however, these methods are expensive and time-consuming. Analytes in complicated samples can now be determined quickly and easily without the need for prior separation thanks to the application of spectrophotometric techniques, mathematical algorithms, and wavelength modification. Therefore, this research aimed to develop a simple and selective UV spectroscopic technique (DDRSD) for the concurrent analysis of these compounds in synthetic mixture.

MATERIALS AND METHODS

Chemicals and Material

Gift samples of DAPA (Purity: 99.6%), LINA (Purity: 101.3%) and METF (Purity: 100.6%) were procured from Healthcare Pvt. Ltd Ahmedabad, India, Elxir Pharma Ahmedabad, India and Chemdyes Corporation, Rajkot, India respectively.

Instruments

All the chemicals, solvents and excipients of Analytical research grade used in the study were obtained from chemdyes corporation, Rajkot, India. UV-1700 with UV Probe Version 2.42 (Shimadzu Double beam UV-visible spectrophotometer) for spectra recording and Electronic balance (Reptech) were utilized for weighing.

Preparation of Stock Solution

DAPA and LINA were transferred separately in a 100 ml volumetric flask by weighing 10 mg of the standard analyte and METF by weighing 100 mg of the standard analyte and then filled up to the mark with methanol. The final solution represents 100 µg/ml of DAPA and LINA and 1000 µg/ml of METF.

Formulation of Synthetic Mixture

It is comprised of DAPA, LINA and METF was prepared in a ratio of 10:5:500 w/w/w. This mixture was combined meticulously

with conventional excipients, including carboxymethyl cellulose (50 mg), talc (15 mg), lactose anhydrous (10 mg), and microcrystalline cellulose (200 mg), and magnesium stearate (10 mg). The pure drugs of DAPA, LINA, and METF were incorporated into the mixture, and all components were thoroughly mixed to ensure homogeneity.

DDRDS

Optimization of variables

Divisor concentration and wavelength

In this method, a divisor consisting of the summation of 2 samples of a ternary mixture was utilized. The saved spectra of the standard solution of DAPA were divided by the sum of LINA and METF, LINA was divided by the sum of DAPA and METF, and METF was divided by the sum of DAPA and LINA, respectively.

For optimization purposes, various combinations of LINA + METF (10 µg/ml + 10 µg/ml, 4 µg/ml + 10 µg/ml), DAPA + METF (5 µg/ml + 5 µg/ml, 10 µg/ml + 10 µg/ml, 20 µg/ml + 20 µg/ml), and LINA + DAPA (10 µg/ml + 10 µg/ml, 4 µg/ml + 10 µg/ml) were tested. Then derivative of the ratio spectra were carried out by using UV Probe version. Selection of the wavelength (maxima or minima) was done on the basis of strong linear response to the concentration of the analyte.

Δλ and scaling factor

To determine the appropriate Δλ value for differentiation, a series of ratio spectra (RS) with different amounts of DAPA (0.5 to 25 µg/ml), LINA (0.2 to 14 µg/ml) and METF (5 to 50 µg/ml) were generated. In each case, 10 µg/ml of (LINA + METF), (METF + DAPA) and (LINA + DAPA) were used as divisors and analyzed under the selected parameters at various Δλ values (5, 10, 20, 40 nm) and at different scaling factors (1, 5, 10).

Method Validation

The developed technique has been validated in accordance with the guidelines of the ICH **[52].**

Linearity

Series A comprises of various concentrations of DAPA ranging from 0.5 – 25 µg/ml. To prepare series A, appropriate volumes (0.5 ml, 5 ml, 10 ml, 15 ml, 20 ml, and 25 ml) were pipetted from a stock solution of DAPA (100 µg/ml) into a set of volumetric flasks (100 ml), and distilled water was added to adjust the volume. Series B includes various amounts of LINA ranging from 0.2 to 14 µg/ml. To prepare Series B, suitable aliquots (0.2 ml, 4 ml, 8 ml, 10 ml, 12 ml, and 14 ml) were pipetted from stock solution of LINA (100 µg/ml) into a series of volumetric flasks (100 ml), followed by addition of distilled water to adjust the volume. Series C consists of various amount of METF from 5 to 50

µg/ml. To prepare Series C, appropriate volumes (0.5 ml, 1 ml, 2 ml, 3 ml, 4 ml, and 5 ml) were withdrawn from standard solution of METF (1000 µg/ml) in a set of volumetric flasks (100 ml) and distilled water was added to adjust the volume. Series D composed of mixture containing varying amounts of DAPA (0.5 – 25 µg/ml), LINA (0.2 – 14 µg/ml), and METF (5 – 50 µg/ml). The absorbance of Series D was measured at 282 nm, 316.74 nm, and 236 nm for assessing DAPA, LINA and METF correspondingly. These measurements were conducted using summations of saved spectra of 10 µg/ml of each of the other two components as divisors. Under the described experimental conditions, the graphical representations of mean absorbance values (n = 3) versus concentration demonstrated a satisfactory linear relationship and a high correlation coefficient (R^2).

Precision

Repeatability

Solutions containing 0.8 µg/ml DAPA, 0.4 µg/ml LINA & 40 µg/ml METF were analyzed six times and the % RSD was calculated.

Intermediate Precision

Intraday and Interday precision was performed by analyzing solution comprising 3 different concentration DAPA/LINA/METF (10/8/20 µg/ml, 15/10/30 µg/ml, 20/12/40 µg/ml) at three time points and on 3 successive days. The % RSD was recorded.

Accuracy

The accuracy was performed using placebo recovery method at three levels (80%, 100%, 120 %). Sample preparation was as shown in **Table 1**.

Table 1: Sample Preparation for Accuracy

Drugs	%Level	Sr. no.	1st dilution			2nd dilution		3rd dilution	
			Wt. of Placebo (mg)	Wt. of API (mg)	Dilute (ml)	Aliquot (ml)	Dilute (ml)	Aliquot (ml)	Dilute (ml)
DAPA	80	1	285	8	100	2	50	10	50
	100	2	285	10	100	2	50	10	50
	120	3	285	12	100	2	50	10	50
LINA	80	1	285	4	100	2	50	10	50
	100	2	285	5	100	2	50	10	50
	120	3	285	6	100	2	50	10	50
METF	80	1	285	400	100	2	50	10	50
	100	2	285	500	100	2	50	10	50
	120	3	285	600	100	2	50	10	50

Limit of detection (LOD) and Limit of quantitation (LOQ)

The equation as follows were utilised to determine LOD and LOQ.

$LOD = 3.3 \times \sigma/S$ $LOQ = 10 \times \sigma/S$

σ = SD of the response (y intercept) and S = slope of the calibration curve

Analysis of sample solution

The synthetic mixture equivalent to one tablet was added to volumetric flask (100

ml). After adding 30 ml of methanol, sonication was done for 20 minutes. Methanol was utilises as diluent. Filtration was done and then 2 ml (filtrate) was added to 50 ml volumetric flask and diluted with distilled water. Furthermore, 10 ml was pipetted in 50 ml volumetric flask and diluted with distilled water to the mark, resulting in solution containing 0.8 µg/ml DAPA, 0.4 µg/ml LINA and 40 µg/ml METF. The parameter was assessed thrice (n = 3). The determination was made for individual analyte at respective wavelength and the amount of each samples was subsequently calculated.

RESULTS AND DISCUSSION

Method Development and Optimization

The overlain Spectra of DAPA (0.5 – 25 µg/ml), LINA (0.2 – 14 µg/ml) and METF (5 – 50 µg/ml) was shown in **Figure 2** and their ternary mixture comprised of varying proportions of DAPA, LINA and METF prepared in distilled water were recorded as shown in **Figure 3** and stored in computer. To optimize the analysis, different concentrations of DAPA, LINA and METF in combination were tested as DDRSD method was proposed. 10 µg/ml for each drug was chosen as the divisor because it gave the most favorable (S/N) and better R^2, indicating the best fit of the data to a linear line. For the quantitation of DAPA and LINA, the output was computed at 282 nm and 316.74 nm correspondingly **(Figure 4 and Figure 5)**. METF was estimated at 236 nm **(Figure 6)**. These wavelengths were selected as they showed the most linear response to analyte concentration. The derivation of the ratio spectra was performed using UV Probe version 2.42 software to ensure accurate and efficient analysis. Different values were tried, $\Delta\lambda$ value = 10 nm and scaling factor = 5 were found as optimal.

Method Validation

The validation of the proposed procedures was conducted following the guidelines outlined by ICH.

Linearity

A linear correlation was observed between output (absorbance) and the amount of DAPA, LINA and METF in the range of 0.5-25 µg/ml, 0.2 –14 µg/ml & 5-50 µg/ml with an R^2 value of 0.9996 for DAPA, 0.9991 for LINA and 0.9994 for METF, respectively **(Table 2)**.

Precision Repeatability

The % RSD values for the repeatability of the ternary mixtures containing DAPA (0.8 µg/ml), LINA (0.4 µg/ml) and METF (40 µg/ml) at 282 nm, 316.74 nm and 236 nm were less than 2 %. The outcome of the parameter are represented in **Table 2**.

Intermediate precision (Intraday and Interday)

Triplicate estimation of three ternary

mixtures comprising DAPA/LINA/METF (10/8/20 µg/ml, 15/10/30 µg/ml, 20/12/40 µg/ml) was performed on same day and on 3 consecutive days. The % RSD was computed for both intraday and interday precision. The outcome of parameters are shown in **Table 2**.

LOD and LOQ

The LOD and LOQ results show that the developed approach is sensitive for the concurrent analysis of DAPA, LINA and METF as shown in **Table 2**.

Accuracy

The parameter was assessed employing the placebo recovery method. The results of the recovery studies were in the range of 98-102 % for each drug, show better recovery of analyte by proposed approach. The outcomes are shown in **Table 3**.

Quantification of DAPA, LINA and METF in Synthetic mixture

The proposed approach was utilised to estimate DAPA, LINA and METF in synthetic mixture. The synthetic mixture was tested three times and the percentage label claim was calculated. The outcome represented in **Table 4** showed that the method is suitable for concurrent quantitation of cited drugs without interference from excipients.

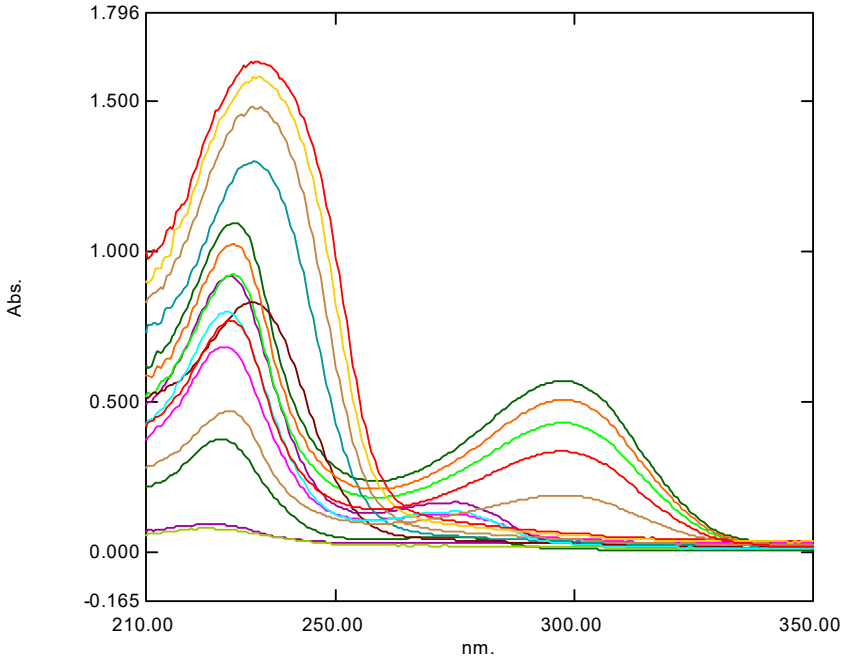

Figure 2: Overlain absorption spectra of DAPA (0.5- 25 µg/ml), LINA (0.2-14 µg/ml) and METF (5-50 µg/ml)

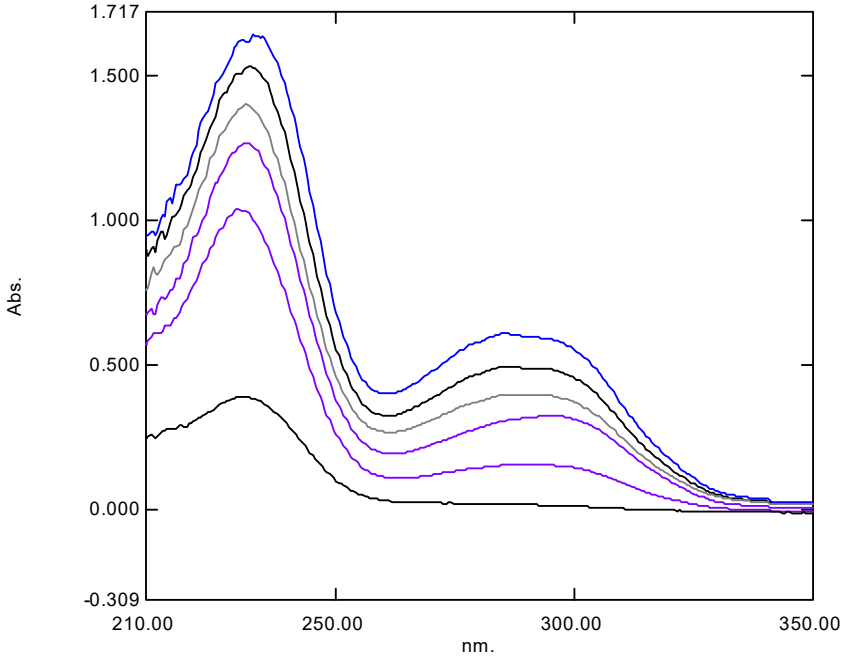

Figure 3: UV spectra of Ternary Mixture

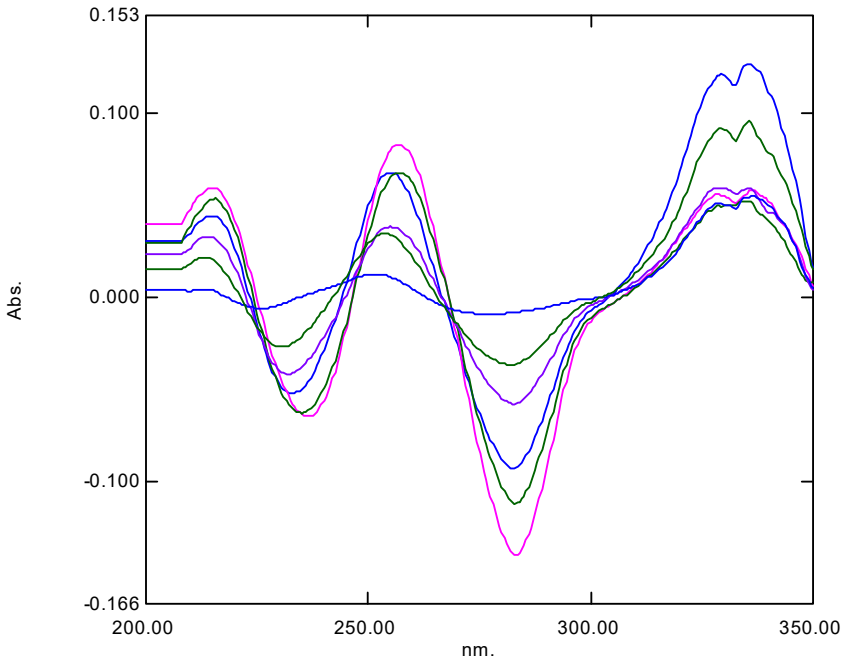

Figure 4: (1st) derivative of ratio spectra of ternary mixture of when METF and LINA 10 µg/ml used as divisor

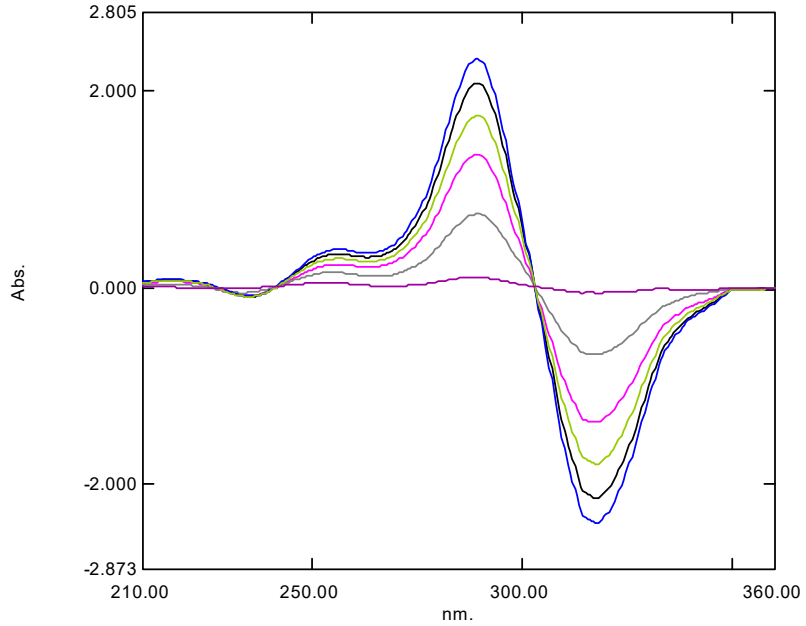

Figure 5: (1st) derivative of ratio spectra of ternary mixture of when METF and DAPA 10 µg/ml used as divisor

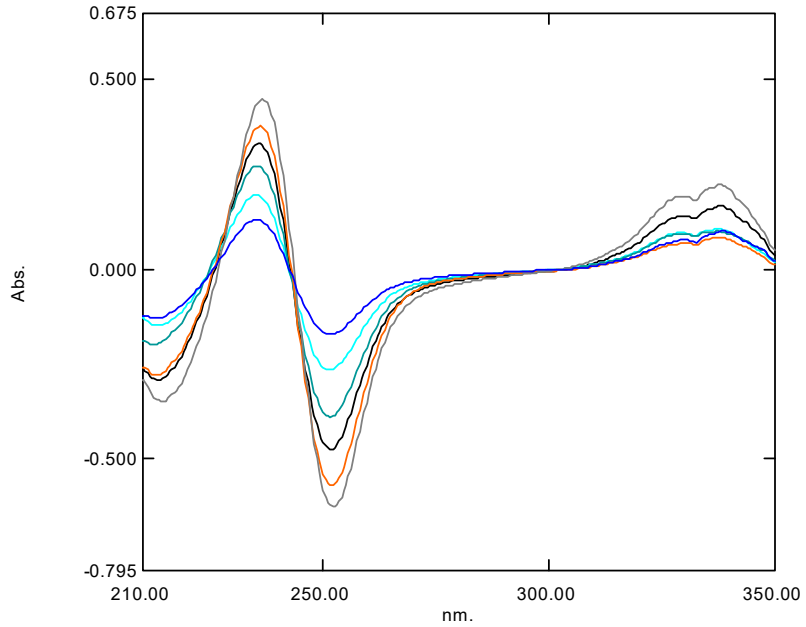

Figure 6: (1st) derivative of ratio spectra of ternary mixture of when LINA and DAPA 10 µg/ml used as divisor

Table 2: Results of validation parameters of DAPA, LINA and METF

Parameters/Drugs	Results		
	DAPA	LINA	METF
Wavelength (nm)	282	316.74	236
Linearity (µg/ml)	0.5-25	0.2-14	5-50
Correlation co-efficient (R^2)	0.9996	0.9991	0.9994
Regression equation	y = 0.004x + 0.0091	y = 0.1432x + 0.0284	y = 0.0102x + 0.0255
Limit of detection (µg/ml)	0.16	0.05	0.23
Limit of quantitation (µg/ml)	0.25	0.10	0.72
Precision (% RSD)			
Repeatability (n=6)	1.68	0.76	1.37
Intraday (n=3)	0.24 - 1.01	0.25 - 0.94	0.52 - 0.91
Interday (n=3)	0.22 - 0.96	0.98 - 1.32	0.24 - 0.89

Table 3: Recovery Result

Drugs	(%) Recovery Level	(%) Recovery ± SD
DAPA	80	99.0 ± 0.461
	100	100.6 ± 1.664
	120	99.9 ± 1.530
LINA	80	99.5 ± 1.568
	100	100.3 ± 1.443
	120	99.8 ± 1.159
METF	80	100.6 ± 1.004
	100	100.1 ± 1.092
	120	99.8 ± 1.167

Table 4: Result of synthetic mixture analysis

Drugs	Label claim	Amount Found (n=3)	(%) Purity ± SD (n=3)	(%) RSD
DAPA	10 mg	9.8 mg	99.6 ± 1.15	1.15
LINA	5 mg	5.3 mg	101.3 ± 0.57	0.56
METF	500 mg	502 mg	100.6 ± 1.52	1.51

CONCLUSION

The combination of DAPA, LINA and METF was employed for T2D. A novel, precise, simple and sensitive method was developed and validated in accordance with ICH guidelines for the determination of three drugs in a mixture. The proposed DDRSD method can estimate three drugs without interference from excipients and it can resolve the spectral overlaps without the need for prior separation. One additional benefit of using the ratio spectra approach instead of the zero-crossing derivative method is that measurements can be made in accordance with peaks, which could lead to increased sensitivity and accuracy. Therefore, the proposed method is appropriate for routine QC analysis of dosage form and ternary mixtures comprising three cited drugs.

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CONFLICT OF INTEREST

There are no conflicts of interest to declare.

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