# Development and Validation of an RP-HPLC Method for NDMA Determination in Metformin and Ranitidine APIs and Pharmaceutical Formulations

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**Abstract**

A sensitive and fast reverse-phase high-performance liquid chromatography (RP-HPLC) assay was designed and tested to estimate the N-nitrosodimethylamine (NDMA) produced during stress degradation of metformin and ranitidine in active pharmaceutical ingredients (APIs) and pharmaceutical formulations quantitatively. Using a C18 column and a mobile phase of acetonitrile and ammonium phosphate buffer (20 mM) in a ratio of 10:90 (v/v), chromatographic separation was accomplished under isocratic conditions. The pH was corrected to 4.11 using 0.1% formic acid. Analysis was carried out at room temperature while the flow rate was kept at 1.0 mL/min. With a correlation coefficient ($r^2$) of 0.9919, the method showed linearity over the concentration range of 0.01–0.1 µg/mL.

The limit of detection and limit of quantification were found to be 0.0152 µg/mL and 0.0461 µg/mL, respectively. Intra-day and inter-day precision results were within acceptable limits, indicating good repeatability of the method. Robustness studies showed no significant variations upon deliberate changes in pH, flow rate, and detection wavelength. Stress degradation studies were carried out under acidic (0.1 N HCl), alkaline (0.1 N NaOH), and oxidative (3% $H_2O_2$) conditions. The developed method was found to be suitable for the direct estimation of NDMA in metformin and ranitidine APIs and formulations during stress degradation studies.

**Keywords**

N-nitrosodimethylamine; Metformin; Ranitidine; Stress degradation studies; RP-HPLC

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## 1. INTRODUCTION

Nitrosamine impurities have emerged as a major concern in the pharmaceutical industry due to their potent genotoxic and carcinogenic properties. Until recently, nitrosamine impurity testing was not routinely mandated in pharmacopoeial standards. However, regulatory authorities such as the United States Food and Drug Administration (USFDA) have reported the presence of nitrosamines in several commonly prescribed medications, leading to large-scale recalls worldwide. These include angiotensin II receptor blockers (ARBs) such as losartan, valsartan, and irbesartan; antihistaminic drugs including ranitidine and nizatidine; and antidiabetic agents such as metformin, all of which were found to contain unacceptable levels of nitrosamine impurities [1–6].

Ranitidine-based products, notably the brand Zantac, and extended-release formulations of metformin have been recalled owing to the detection

of N-nitrosodimethylamine (NDMA), a highly toxic nitrosamine impurity [7–10]. These drugs are widely used in the management of gastric ulcers and as first-line therapy for type 2 diabetes mellitus, respectively. The presence of NDMA in pharmaceutical products poses a significant public health risk, as nitrosamines are known to induce carcinogenesis in multiple organs, including the stomach, esophagus, and pharynx [11–13].

Among various nitrosamines, NDMA (N-nitrosodimethylamine), also known as N,N-dimethylnitrous amide, has gained particular attention due to its frequent occurrence and high carcinogenic potential. NDMA has the molecular formula $C_2H_6N_2O$ and is primarily formed through the reaction of secondary or tertiary amines with nitrosating agents such as sodium nitrite or nitrous acid, especially under acidic conditions. Additionally, the use of nitrous acid for quenching residual azides during pharmaceutical synthesis can further increase the risk of nitrosamine formation [14–19]. The chemical structures of NDMA, metformin, and ranitidine are illustrated in Fig. 1.

Several advanced analytical techniques have been reported in the literature for the determination of nitrosamines in pharmaceutical substances and formulations, including liquid chromatography–high-resolution mass spectrometry (LC-HRMS) and gas chromatography–tandem mass spectrometry (GC-MS/MS). Hyun-Hee Lim et al. described a solid-phase extraction GC-MS/MS method for the determination of NDMA in sartans, metformin, and ranitidine, reporting limits of detection and quantification of 0.3 µg/mL and 0.07 µg/mL, respectively. Regulatory agencies have also reported LC-ESI-HRMS methods capable of detecting NDMA at ng/mL levels, with reported linearity ranges of 1.0–10 ng/mL [20–22]. Despite their high sensitivity, these techniques require sophisticated instrumentation and are not always suitable for routine quality control laboratories.

In this context, the present study aims to develop and validate a sensitive, rapid, and cost-effective reverse-phase high-performance liquid chromatography (RP-HPLC) method for the estimation of NDMA in metformin and ranitidine active pharmaceutical ingredients (APIs) and pharmaceutical formulations under stress degradation conditions. The method's linearity, accuracy, robustness, limit of detection, and limit of quantification were all validated in compliance with International Council for Harmonization (ICH) recommendations. Furthermore, the applicability of the developed method was demonstrated through the analysis of NDMA formation in commercially available APIs and formulations subjected to stress degradation studies.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals and reagents

HPLC-grade acetonitrile, HPLC-grade methanol, ammonium formate, formic acid, potassium bromide (for IR spectroscopy), and HPLC-grade water were used throughout the study. N-nitrosodimethylamine (NDMA) standard solution (200 µg/mL in methanol) was procured from Sigma-Aldrich. Metformin and ranitidine active pharmaceutical ingredients (APIs) were obtained from Pure and Health Care Private Limited, Raipur, India. Metformin hydrochloride extended-release tablets (Carbophage XR 500) were purchased from Messia Pharmacy, Nilgiri, India, manufactured by Merck. Ranitidine hydrochloride tablets were procured from Tamil Nadu Government Supply, manufactured by Vivek Pharmachem (India) Ltd., Jaipur, India.

### 2.2. Instrumentation

Chromatographic analysis was performed using a Shimadzu Prominence HPLC system equipped with a UV detector, high-pressure binary pumps, and a Rheodyne manual injector fitted with a 20 µL sample loop. Separation was achieved using a Hibar C18 column (250 × 4.6 mm i.d., 5 µm particle size). All analyses were carried out at ambient temperature. Data acquisition and processing were performed using LabSolutions software.

### 2.3. Chromatographic conditions

Isocratic elution was employed using a mobile phase composed of acetonitrile and ammonium phosphate buffer (20 mM) in the ratio of 10:90 (v/v). The pH of the buffer was adjusted to 4.11 using 0.1% formic acid. A wavelength of 245 nm was used for detection, and the flow rate was kept constant at 1.0 mL/min.

### 2.4. Preparation of solutions

#### *2.4.1. Preparation of buffer solution*

Ammonium formate buffer (20 mM) was prepared by dissolving 1.26 g of ammonium formate in 1000 mL of HPLC-grade water. After passing the mixture through a 0.45 µm membrane filter, 0.1% formic acid was used to bring the pH down to 4.11. The buffer was sonicated for 5 min prior to use.

#### *2.4.2. Preparation of standard solutions*

The NDMA standard solution (10 µg/mL) was prepared by diluting 0.5 mL of the 200 µg/mL stock solution to 10 mL with HPLC-grade methanol, followed by sonication for 3 min.

Metformin standard solution (10 µg/mL) was prepared by accurately weighing 10 mg of metformin and dissolving it in a 10 mL volumetric flask using the mobile phase as diluent to obtain a concentration of 1000 µg/mL. Appropriate dilutions were made to achieve a final concentration of 10 µg/mL, followed by sonication for 3 min. Ranitidine standard solution (10 µg/mL) was prepared in a similar manner by dissolving 10 mg of ranitidine in a 10 mL volumetric flask using the mobile phase as diluent, followed by further dilution and sonication.

#### *2.4.3. Preparation of sample solutions*

For metformin formulation, three tablets were weighed, finely powdered using a mortar and pestle, and the average tablet weight was calculated. An amount equivalent to the average tablet weight was transferred into a 100 mL beaker, and 50 mL of diluent was added. Whatman filter paper was used to filter the mixture after it had been sonicated for fifteen minutes. The filtrate was transferred to a 100 mL volumetric flask and the volume was made up with diluent. Based on the label claim of 500 mg per tablet, the resulting solution contained 500 µg/mL of metformin, which was further diluted to obtain a concentration of 10 µg/mL.

For ranitidine formulation, three tablets were weighed and powdered. An amount equivalent to the average tablet weight was transferred into a 100 mL beaker, dissolved in 50 mL of diluent, sonicated for 15 min, and filtered. The volume was adjusted to 100 mL with diluent. Based on the label claim of 150 mg per tablet, the resulting solution contained 1500 µg/mL of ranitidine, which was further diluted to obtain a final concentration of 10 µg/mL.

### 2.5. Method validation

The International Council for Harmonization (ICH) guidelines were followed in the validation of the developed RP-HPLC method. [23–26]. Linearity was evaluated over a concentration range of 0.01–0.1 µg/mL for NDMA using nine calibration levels (0.01, 0.02, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, and 0.1 µg/mL). Plotting peak area against concentration allowed for the construction of calibration curves and the determination of the correlation coefficient and linear regression equation. The equations LOD = 3.3σ/S and LOQ = 10σ/S were used to compute the limit of detection (LOD) and limit of quantification (LOQ) based on the slope (S) of the calibration curve and the standard deviation of the response (σ). Intra-day and inter-day variations were used to gauge precision.

Intra-day precision was evaluated by injecting NDMA standard solution (10 µg/mL) at different time intervals on the same day, while inter-day precision was determined by injecting the same concentration on different days. The percentage relative standard deviation (%RSD) was calculated for each case.

To assess the method's robustness, intentional modifications were made to the chromatographic settings, such as the detection wavelength (±2 nm), flow rate (±0.1 mL/min), buffer pH (±0.2), and column oven temperature. Ruggedness was evaluated by performing the analysis under different conditions, including analysis by different analysts, use of different HPLC instruments within the same laboratory, use of different C18 columns (Hibar C18, Princeton Sphere C18, and Phenomenex Luna C18), and use of reagents and solvents obtained from different manufacturers.

System suitability testing was performed to evaluate chromatographic performance parameters such as resolution, column efficiency (number of theoretical plates), peak asymmetry factor, and percentage relative standard deviation. The difference in the retention periods of neighboring peaks was used to calculate resolution (Rs). The formula $N = 16 (Rt/W)^2$ was used to determine column efficiency (N), where W is the peak width and Rt is the retention time. The ratio of the distances on either side of the peak midpoint was used to compute the peak asymmetry factor. Six replicate injections of the NDMA standard solution were used to calculate the %RSD.

### 2.6. Stress degradation studies

Stress degradation studies were performed on metformin and ranitidine APIs and formulations under acidic, alkaline, and oxidative conditions in accordance with ICH guidelines. Acidic degradation was carried out by exposing the samples to 0.1 N hydrochloric acid for 0, 3, 5, 24, and 48 h under optimized chromatographic conditions, followed by chromatographic analysis.

Alkaline degradation was performed by treating the samples with 0.1 N sodium hydroxide for 0, 3, 5, 24, and 48 h, and the resulting solutions were analyzed under the same chromatographic conditions. Oxidative degradation was conducted by exposing the samples to 3% hydrogen peroxide for 0, 3, 5, 24, and 48 h, followed by chromatographic analysis.

## 3. RESULTS AND DISCUSSION

### 3.1. Selection of detection wavelength

The ultraviolet absorption spectrums of N-nitrosodimethylamine (NDMA), metformin, and ranitidine were measured at 200-400nm in 10 5g/mL dilution. NDMA possessed a maximum absorbance

( λmax ) at 229 nm, whereas the metformin and ranitidine had λmax at 232 nm and 228 nm, respectively. The overlay spectra of the three analytes showed an isosbestic point at 245 nm, which implies that there was low spectral interference between the compounds. Thus, the detection wavelength of 245 nm was chosen to further perform chromatographic analysis because it would offer sufficient sensitivity and selectivity of NDMA in the presence of metformin and ranitidine.

### 3.2. Method optimization

The RP-HPLC method was optimized according to the physicochemical properties of the analytes such as polarity, ionization behavior and chromatographic retention. The column was a Hibar C18 column (250 x 4.6 mm i.d., 5 mm) which was chosen to have effective separation. A 10:90 (v/v) ratio of acetonitrile and ammonium phosphate buffer constituted the optimum mobile phase and 0.1% formic acid was added to reduce the pH to 4.11. Isocratic elution at a flow rate of 1.0 mL/min and a demonstration of 245 nm gave well-separated and symmetrical peaks. In these optimized conditions NDMA, metformin and ranitidine retention times were established as 5.031min, 3.049min and 10.835min respectively. The appropriateness of the methodology in selective estimation of NDMA in the presence of metformin and ranitidine was confirmed by adequate resolution of NDMA with the parent drugs as demonstrated in Fig. 2.

### 3.3. Linearity

Standard NDMA solutions were used to test the linearity of the developed method within the concentration range, 0.01 to 0.1 µg/mL. The linearity of the calibration curve was shown by the correlation of the peak area and the concentration with a correlation coefficient (r 2) of 0.9919. The regression equation that was obtained was y = 2 x 10 6x + 37201, which implies proportional detector response in the studied range (Fig. 3). The experimentally calculated limit of detection (LOD) and limit of quantification (LOQ) of NDMA in 0.0152 µg/mL and 0.0461 µg/mL respectively, are summarized in Table 1.

### 3.4. Precision

Accuracy of the procedure was determined on the basis of intra-day and inter-day range using the NDMA standard solution at the concentration of 10 µg/mL. The intra-day and inter-day relative standard deviation percentages (%RSD) were less than 2, which shows that the method has high repeatability and medium precision. The data on precisions is in Table 2.

### 3.5. Robustness

The developed RP-HPLC method was tested in terms of robustness by adding controlled changes in the chromatographic parameters such as pH of the buffer, flow rate, and wavelength of detection. The retention time and peak response did not vary significantly under the conditions tried, which means that the method is robust and reliable to use on a regular basis. Table 3 summarizes the results of the robustness.

### 3.6. System suitability

System suitability parameters such as resolution, column efficiency (number of theoretical plates), peak asymmetry factor, and percentage relative standard deviation were evaluated prior to analysis. All system suitability criteria were found to be within acceptable limits, confirming the adequacy of the chromatographic system for NDMA analysis. The system suitability results are presented in Table 5. Although the theoretical plate count was marginally below the conventional acceptance criterion of 2000, satisfactory resolution, peak symmetry, and reproducibility confirmed the adequacy of the chromatographic performance for NDMA analysis.

### 3.7. Estimation of NDMA in APIs and formulations by stress degradation studies

Stress degradation studies were performed to evaluate the formation of NDMA in metformin and ranitidine APIs and pharmaceutical formulations under acidic, alkaline, and oxidative conditions.

#### 3.7.1. Acidic degradation

Acidic degradation was carried out by exposing metformin and ranitidine samples to 0.1 N hydrochloric acid for 0, 3, 5, 24, and 48 h. Chromatographic analysis revealed no detectable NDMA peak in either the APIs or formulations under acidic conditions. The representative chromatograms for acid-treated metformin and ranitidine are shown in Fig. 4(a) and Fig. 5(a), respectively.

#### 3.7.2. Alkaline degradation

Alkaline degradation studies were conducted using 0.1 N sodium hydroxide under the same exposure intervals. No NDMA formation was observed in both metformin and ranitidine APIs and formulations following alkaline treatment. The corresponding chromatograms are presented in Fig. 4(b) and Fig. 5(b).

#### 3.7.3. Oxidative degradation

Oxidative degradation was performed by treating the samples with 3% hydrogen peroxide for 0, 3, 5, 24, and 48 h. Chromatographic evaluation showed no detectable NDMA peaks in either APIs or formulations under oxidative stress conditions. The chromatograms for oxidative degradation of metformin and ranitidine are shown in Fig. 4(c) and Fig. 5(c), respectively.

The absence of NDMA formation under all tested stress conditions indicates that metformin and ranitidine, in both API and formulation forms, did not generate NDMA under the applied degradation conditions. This further demonstrates the specificity and suitability of the developed RP-HPLC method for NDMA monitoring in pharmaceutical products.

## 4. CONCLUSION

A sensitive and reliable RP-HPLC method was successfully developed and validated for the estimation of N-nitrosodimethylamine (NDMA) in metformin and ranitidine active pharmaceutical ingredients and pharmaceutical formulations under stress degradation conditions. The method demonstrated satisfactory precision, accuracy, specificity, robustness, and sensitivity, with acceptable limits of detection and quantification, in accordance with ICH validation guidelines.

Stress degradation studies conducted under acidic (0.1 N HCl), alkaline (0.1 N NaOH), and oxidative (3% $H_2O_2$) conditions confirmed effective chromatographic separation of NDMA from metformin and ranitidine, with a total analysis time of 13 min. No NDMA formation was detected in either the APIs or marketed formulations under the applied stress conditions. Overall, the developed RP-HPLC method is suitable for routine quality control and stability studies for monitoring NDMA impurities in metformin and ranitidine pharmaceutical products and provides a practical, cost-effective alternative to advanced mass spectrometry-based techniques.

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**Figure and Table Legends**

**Fig. 1. Chemical structures of (a) N-nitrosodimethylamine (NDMA), (b) metformin, and (c) ranitidine.**

**Fig. 2. Representative RP-HPLC chromatograms of (a) ranitidine, (b) metformin, and (c) N-nitrosodimethylamine (NDMA) under optimized chromatographic conditions.**

**Fig. 3. Calibration (linearity) curve of N-nitrosodimethylamine (NDMA) in the concentration range of 0.01–0.1 µg/mL.**

**Fig. 4. RP-HPLC chromatograms of stress degradation of metformin under (a) acidic (0.1 N HCl), (b) alkaline (0.1 N NaOH), and (c) oxidative (3% $H_2O_2$) conditions.**

**Fig. 5. RP-HPLC chromatograms of stress degradation of ranitidine under (a) acidic (0.1 N HCl), (b) alkaline (0.1 N NaOH), and (c) oxidative (3% $H_2O_2$) conditions.**

**Table 1. Linearity and calibration range of N-nitrosodimethylamine (NDMA).**

**Table 2. Intra-day and inter-day precision of the developed RP-HPLC method.**

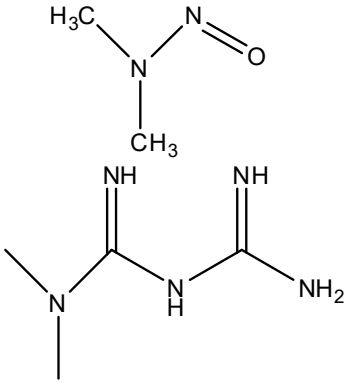
**Table 3. Limit of detection (LOD) and limit of quantification (LOQ) of N-nitrosodimethylamine (NDMA).**

**Table 4. Robustness of the developed RP-HPLC method under deliberate variations in chromatographic conditions.**

**Table 5. System suitability parameters of the developed RP-HPLC method.**

**Figures**

(a) (b)

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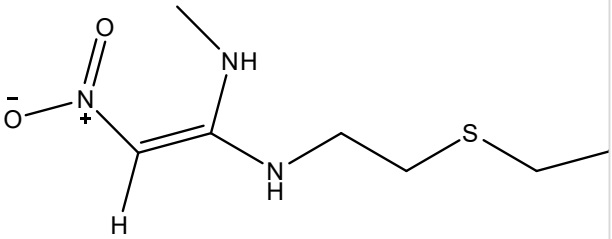



**Fig.1.**

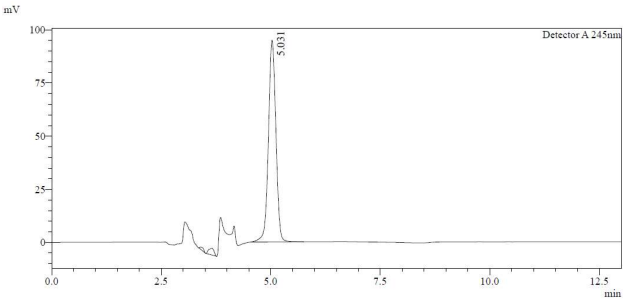



**Fig.2.**

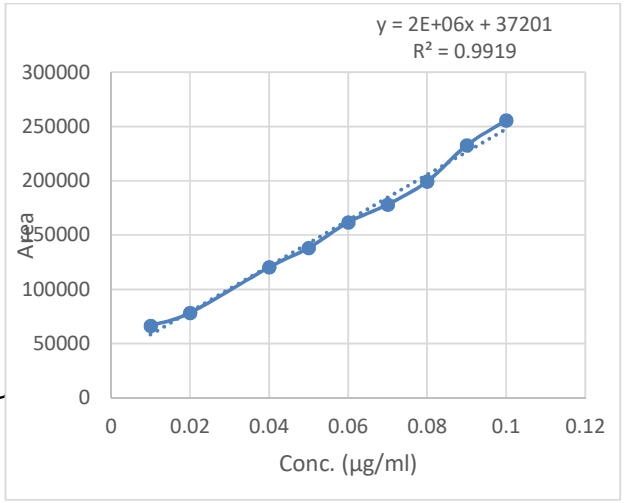
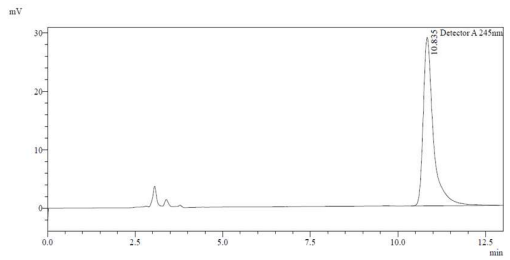



**Fig.3.**

(a)

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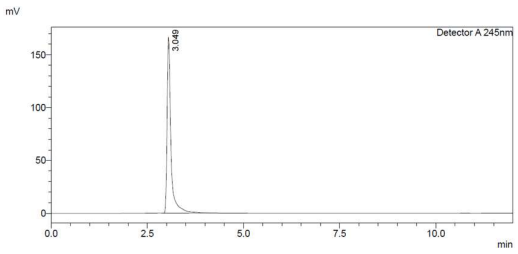
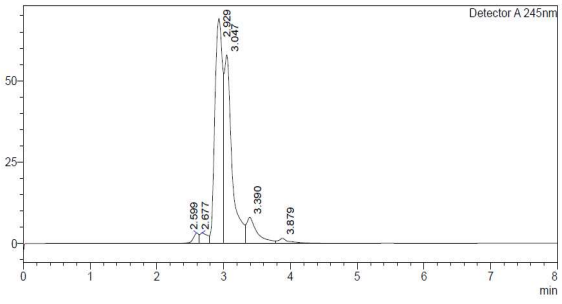



(c)

(a)

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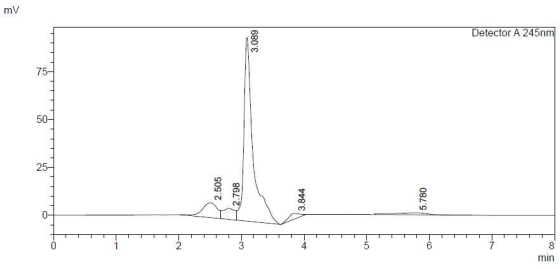

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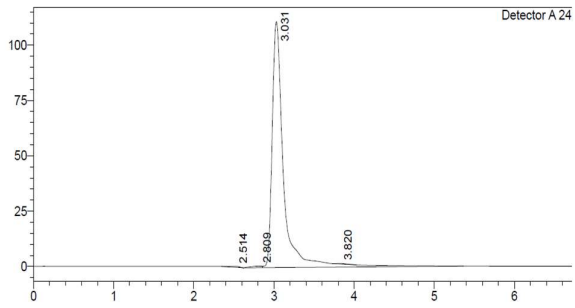
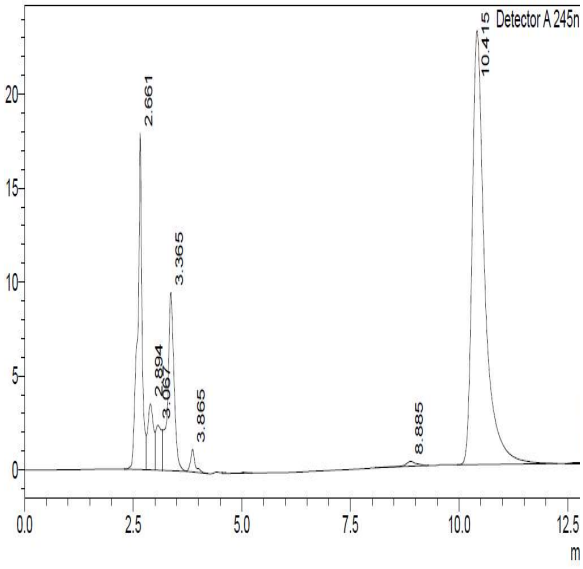



**Fig.4.**

(a)

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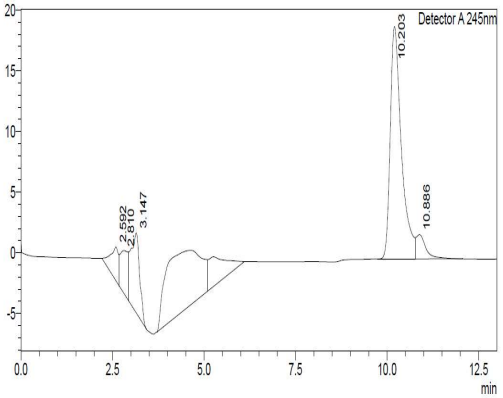


(c)

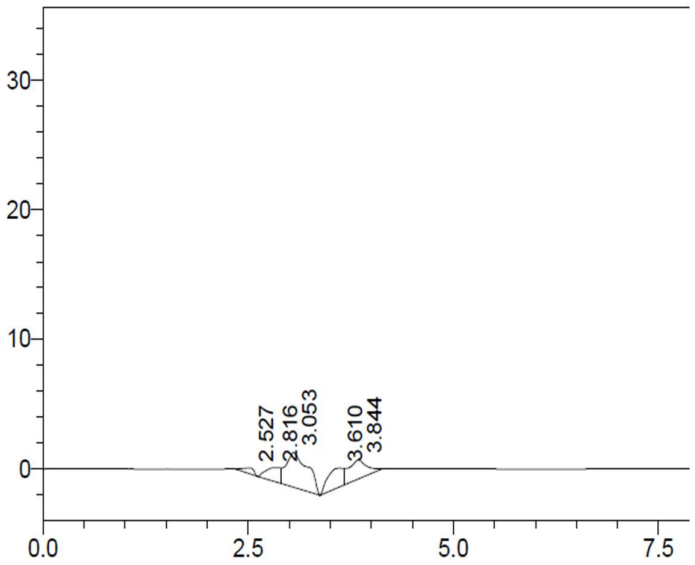


**Fig.5.**

**Tables**

Table 1

| Concentration (µg/ml) | Peak Area |
|---|---|
| 0.01 | 66386 |
| 0.02 | 78291 |
| 0.04 | 120255 |
| 0.05 | 138104 |
| 0.06 | 161649 |
| 0.07 | 178144 |
| 0.08 | 199346 |
| 0.09 | 232661 |
| 0.1 | 255797 |

Table 2

| | Intra- day | Inter- day |
|---|---|---|
| **Average peak area (n=5)** | 1121201 | 1123802.5 |
| **% RSD** | 0.39 | 0.59 |

Table 3

| Name of compound | LOD (µg/ml) | LOQ (µg/ml) |
|---|---|---|
| NDMA | 0.0152 | 0.0461 |

Table 4

| | pH | | | Flow rate (ml/min) | | | Detector wavelength (nm) | | |
|---|---|---|---|---|---|---|---|---|---|
| | **3.90** | **4.11** | **4.20** | **0.8** | **1** | **1.2** | **244** | **245** | **246** |
| **RT (min)** | 4.812 | 5.031 | 5.131 | 6.613 | 5.031 | 4.569 | 6.354 | 5.031 | 4.418 |

Table 5

| Parameters | Values |
|---|---|
| Resolution between NDMA and Metformin | 3.58 |
| Resolution between NDMA and Ranitidine | 4.33 |
| Column efficiency | 1779.8846 |
| Peak asymmetric factor | 1.007 |
| %RSD of NDMA Peak area response | 0.39 |