Simple and Sensitive RP-HPLC and UV Spectroscopic Methods for the Determination of Remogliflozin Etabonate in Pure and Pharmaceutical Formulations

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SIMPLE AND SENSITIVE RP-HPLC AND UV SPECTROSCOPIC METHODS FOR THE DETERMINATION OF REMOGLIFLOZIN ETABONATE IN PURE AND PHARMACEUTICAL FORMULATIONS

SAF VE FARMASÖTIK FORMÜLASYONLARDA REMOGLIFLOZIN ETABONAT TAYINI İÇIN BASIT VE HASSAS RP-HPLC VE UV SPEKTROSKOPIK YÖNTEMLER

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ABSTRACT
Objectives: Simple, novel and selective RP-HPLC and UV spectroscopic methods have been developed and optimized for the determination of remogliflozin etabonate (RMZ) in bulk and dosage forms.
Materials and Methods: In HPLC method, the principal peak and internal standard peak were eluted separately at different retention times with the chromatographic conditions such as, mobile phase consists of 0.02 M ammonium acetate buffer (pH was adjusted to 4.0 by 1.0 M ortho phosphoric acid), acetonitrile and tetrahydrofuran (THF) in the ratio 50:45:05, respectively (v/v) and the stationary phase used was C18, 5 µ.m, 4.6 mm x 250 mm Kromasil column. The flow rate was 2.0 $mLmin^{-1}$, sample

injection volume was 10 µL and the wavelength of detection was fixed at 228 nm. In case UV spectroscopic method, the RMZ was diluted with pure ethanol. The RMZ showed maximum absorbance at 228nm. Hence throughout analysis 228 nm was used for the determination of RMZ.
Results: The retention times of RMZ and internal standard, atorvastatin (ATST) were 6.2 min and 7.0 min, respectively. The resolution between the peaks was found to be more than 2.0. The total run time was fixed at 10 minutes. The linearity range for RP-HPLC method was found to be 10 μgmL^{-1} to 50 μgmL^{-1}, at a fixed concentration of ATST. The linearity range for the UV spectroscopic method was found to be in the range of 100 to 250 μgmL^{-1}. Regression coefficients (R^2) were found above 0.999 for both the techniques. The limit of detection and limit of quantification for RMZ were found to be 1.0 μgmL^{-1} and 3.5 μgmL^{-1} respectively, in RP-HPLC method and 10.0 $\mu gmL^{-1}$and 40 μgmL^{-1}, respectively in UV spectroscopic method.
Conclusion: The developed methods were found to be simple, accurate, reproducible and precise. The RMZ can be analyzed in dual techniques, i.e., chromatographic as well as UV spectroscopic methods for its routine analysis.

Key words: Remogliflozin etabonate, RP-HPLC, UV spectroscopy, bulk and dosage forms

ÖZ

Amaçlar: Remogliflozin etabonatın (RMZ) toplu ve dozaj formlarında belirlenmesi için basit, yeni ve seçici RP-HPLC ve UV spektroskopik yöntemler geliştirilmiş ve optimize edilmiştir.
Gereç ve Yöntemler: HPLC yönteminde, ana pik ve dahili standart pik, mobil faz 0.02 M amonyum asetat tamponundan oluşması gibi kromatografik koşullarla farklı tutma sürelerinde ayrı ayrı elüte edildi (pH, 4.0 x 1.0 M orto fosforik asit olarak ayarlandı.), sırasıyla 50:45:05 oranında (v / v) asetonitril ve tetrahidrofuran (THF) ve kullanılan sabit faz C18, 5 um, 4.6 mm x 250 mm Kromasil kolonu idi. Akış hızı 2.0 mLmin-1, numune enjeksiyon hacmi 10 uL ve saptamanın dalga boyu 228 nm'de sabitlendi. UV spektroskopik yöntem durumunda, RMZ saf etanol ile seyreltildi. RMZ, 228 nm'de maksimum emicilik gösterdi. Bu nedenle, RMZ'nin belirlenmesi için analiz boyunca 228 nm kullanıldı.
Sonuçlar: RMZ ve dahili standart, atorvastatinin (ATST) alıkonma süreleri sırasıyla 6.2 dakika ve 7.0 dakikaydı. Zirveler arasındaki çözünürlük 2.0'dan fazla bulundu. Toplam çalışma süresi 10 dakika olarak sabitlendi. RP-HPLC yöntemi için doğrusallık aralığı, sabit bir ATST konsantrasyonunda 10 µgmL-1 ila 50 µgmL-1 olarak bulundu. UV spektroskopik yöntem için doğrusallık aralığı 100 ila 250 µgmL-1 aralığında bulundu. Her iki teknik için de regresyon katsayıları (R2) 0.999'un üzerinde bulundu. RMZ için tespit limiti ve ölçüm limiti RP-HPLC yönteminde sırasıyla 1.0 µgmL-1 ve 3.5 µgmL-1 ve UV spektroskopik yöntemde 10.0 µgmL-1 ve 40 µgmL-1 olarak bulundu.
Sonuç: Geliştirilen yöntemlerin basit, doğru, tekrarlanabilir ve kesin olduğu görülmüştür. RMZ, rutin analizi için ikili tekniklerle, yani kromatografik ve UV spektroskopik yöntemlerle analiz edilebilir.

Anahtar kelimeler: Remogliflozin etabonat, RP-HPLC, UV spektroskopisi, yığın ve dozaj formları

INTRODUCTION

Remogliflozin etabonate (RMZ) (**Figure 1**) chemically known as (5-Methyl-4-[4-(1-methylethoxy)benzyl]-1-(1-methylethyl)-1H-pyrazol-3-yl 6-O-(ethoxycarbonyl)-β-D glucopyranoside), belongs to the gliflozin category. It is a pro-drug of gliflozin which is used mainly for the non-alcoholic steatohepatitis as well as type-2 diabetes. RMZ helps to reduce the sodium-glucose, transport proteins and also it is accountable for glucose re-inclusion in the kidney.[1,2]

Thorough literature review revealed that few methods were developed and validated by different analytical instruments for the determination of RMZ. [1, 3-10] An analytical method has been developed by UPLC in bulk as well as in formulations for the simultaneous estimation of RMZ and metformin hydrochloride. The mobile phase used was phosphate buffer (pH - 4.5) and acetonitrile in the ratio 60:40 v/v.[1] An UV spectrophotometric method was developed for the simultaneous estimation of empagliflozin and metformin hydrochloride in bulk and dosage forms. [3] The method showed that there were two methods A and B, in method A the absorption was measured at 272 nm and 234 nm for empagliflozin and metformin hydrochloride, respectively. Method B used the absorbance ratio (Q-analysis) in which the absorbance was measured at 254 nm and 226 nm for empagliflozin and metformin hydrochloride, respectively.[3] A liquid chromatographic method was developed and validated for simultaneous estimation of metformin, pioglitazone and glimepiride in dosage forms. [4] This method determined the diabetic drugs except RMZ. RP-HPLC method has been developed for simultaneous determination of dapagliflozin and saxagliptin in the bulk and pharmaceutical dosage forms.[5] A stability indicating HPLC method was developed for the determination of saxagliptin and metformin in the bulk forms.[6] A RP-HPLC method was developed and validated for the simultaneous determination of metformin and saxagliptin in the formulations.[7] The simultaneous estimation of metformin hydrochloride and canagliflozin by stability-indicating RP-HPLC method was developed by Kommineni et al.[8] An assay method was developed and validated for simultaneous determination of metformin hydrochloride and canagliflozin by RP-HPLC instrument.[9] Ayoub developed spectrophotometric and chemometric methods for the simultaneous determination of empagliflozin and metformin in the pharmaceutical formulations.[10] An UV derivative spectrophotometric method was developed for the simultaneous determination of metformin and Remogliflozin by Mahesh et al. [11] A thorough statistical data analysis of the reported methods and proposed methods is given in **Table 1**. The reported methods were not simple in a way that they have a long run time or having a complicated mobile phase. The UPLC instrument is sophisticated but expensive so that all the small scale industries and laboratories cannot afford. Keeping these points in view, we proposed the RP-HPLC and UV spectroscopic methods for the determination of RMZ in bulk and formulations. The results of the proposed methods indicated that the HPLC and UV spectroscopic methods for the determination of RMZ in pure and dosage forms are simple, accurate and rugged. The RP-HPLC method is developed with ATST as an internal standard. For the proposed UV spectroscopic method, the absorbance was measured at 228 nm. Both the methods were validated according to the ICH guidelines.[12]

MATERIALS AND METHODS

Instruments

The proposed method was developed and validated using Shimadzu prominence-i HPLC which consists of an auto-injector, UV detector with a deuterium lamp as the source of light and a quaternary pump. The output signal and chromatographic data were processed by using Lab solution software. Eutech pH meter was used for measuring the pH of the buffer solution. Ultrasonic sonicator bath was used to degas the solvents and nylon membrane of 0.45µm filter paper was used for filtration.

For UV-spectroscopic method, Agilent UV-Visible spectrophotometer (carry 60 model) which consists of a deuterium lamp as a source of light was employed. The spectra were monitored and processed by Win lab software. The solvents used in the experiment were degassed by using the ultrasonic bath.

Chemicals and reagents

RMZ and ATST compounds (>98% purity) were provided by Karnataka antibiotics and pharmaceutical Ltd. Bengaluru, India as gift samples. HPLC grade ammonium acetate, tetrahydrofuran and ethanol (99.9% purity) were purchased from SD finechem Ltd. India. Acetonitrile and ortho phosphoric acid were procured from Merk Ltd, India. The ultra-purified water was prepared by Siemens purifier instrument, India. Column Kromasil, C18, 5 µ.m, 4.6 mm x 250 mm, was obtained from Waters Ltd. For UV spectroscopic method development, pure ethanol was used as diluent.

Preparation of mobile phase, standard solutions and dilutions

The mobile phase was prepared by mixing 0.02 M ammonium acetate buffer (pH adjusted to 4.0 using 1.0 M ortho phosphoric acid), acetonitrile and THF, in the ratio 50:45:05, respectively (v/v). The standard solution was prepared by transferring accurately weighed 100 mg of RMZ to 100 mL standard flask, followed by making up to the mark with the mobile phase. The concentration of the resultant stock solution was 1000 μgmL^{-1}. From this stock solution 0.1 mL of solution was pipetted out into another 100 mL standard flask and made up to the mark with the mobile phase. The concentration of the resulting working standard solution was 1.0 μgmL^{-1}. Similarly, to obtain a linearity graph, the stock solution was diluted to get the concentrations ranging from 10 to 50 μgmL^{-1}. A 30 μgmL^{-1} of internal standard (ATST) was prepared in the mobile phase. For the UV spectroscopic method development, a similar procedure was followed. The standard stock solution and working standard solutions were prepared by taking ethanol as the diluent.

Chromatographic conditions

The mobile phase was composed of buffer solution consisting of 0.02 M ammonium acetate buffer (pH adjusted to 4.0 with 1.0 M ortho phosphoric acid), acetonitrile and tetrahydrofuran (THF) in the ratio 50:45:05, respectively (v/v).The flow rate of the mobile phase was kept at 1.0 $mLmin^{-1}$. The column temperature was kept at 25 ^{0}C and the stationary phase was column Kromasil, C18, 5µ.m, 4.6 mm x 250mm. The wavelength of detection was fixed at 228 nm. The sample injection volume was 10µL. The retention times (RT) of RMZ and ATST were 6.2 minutes and 7.0 minutes respectively.

Spectroscopic conditions

The stock solution of RMZ was scanned between 200 nm - 400 nm which showed maximum absorbance at 228 nm by UV spectrophotometer. Further to confirm the analysis, different concentrations of RMZ drug solutions were scanned. The source of the detector contains a deuterium lamp and quartz cuvettes were used as sample holders.

RESULTS

Method development

The mobile phase equilibrium was primarily conceded using stationary phase column (Kromasil) C18, 5µ.m, 4.6 mm x 250mm. Initially, the mobile phase used for different trials was ammonium acetate and acetonitrile with different concentrations and ratios. In another experiment, 0.02 M ammonium acetate buffer (pH adjusted to 4.0 with 10% dilute acetic acid) and methanol in the ratio 50:50 was tried. In this trial, it was possible to detect peaks but elution was not accurate. Further trials were carried out with different ratios of 0.02 M ammonium acetate (pH - 4.0), acetonitrile and tetrahydrofuran. However, with the mobile phase of ratio 50:45:05, respectively (v/v), the peaks of RMZ and ATST internal standard were eluted with good shape and resolution. Hence, the mobile phase of the ratio 50:45:05 was considered for entire RP-HPLC method development and validation. The flow rate of the mobile phase was kept at 1.0 $mLmin^{-1}$. With these experimental trials, the resulting peaks were eluted satisfactory in accordance with ICH guidelines. In this method, the total run time was 10 minutes for the elution of both peaks. For the detection of eluted peaks, the wavelength of detection was fixed at 228 nm. The proposed method was validated as per the ICH guidelines.[11]

The UV spectroscopic method development was carried out by scanning the RMZ drug in the UV region ranging between 200 nm to 380 nm at different concentrations in the scan mode. The RMZ showed maximum absorbance at 228 nm. Hence λ_{max} of 228 nm was fixed for the entire method development process. The RMZ solution was subsequently diluted with the ethanol to obtain different concentrations according to the desired parameters. All the obtained results are satisfactory and are tabulated in **Table 2**. The parameters were well within the limits as specified in the ICH guidelines.[11]

System suitability

The proposed HPLC method has consistent retention times for RMZ and ATST at 6.2 minutes and 7.0 minutes, respectively. There were no changes in the retention times throughout the analysis. The percentage of related standard deviation (% RSD) from six individual spikes (analytes) was found less than 2.0% at least concentrations, i.e., 0.74 % and 0.82 % for the RMZ and ATST respectively. The system suitability data are tabulated in **Table 2** and the characteristic chromatograms are shown in **Figure 2A**.

The resultant data indicate that the developed method has good sensitivity for RMZ. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 1.0 μgmL^{-1} and 3.5 μgmL^{-1}, respectively and S/N ratio found for LOD and LOQ were 6.5 and 21, respectively. The results were found to be satisfactory and within the limits as shown in **Table 3**.
In the case of the UV spectroscopic method, the percentage of RSD was found to be less than 2.0%. The LOD and LOQ were found to be 10 μgmL^{-1} and 40 μgmL^{-1} respectively. The results were found to be satisfactory and are tabulated in **Table 3**.

Linearity
In UV spectroscopic method development, the five different concentrations of RMZ solutions ranging from 100 to 250 μgmL^{-1} were scanned using a UV spectrophotometer. The RMZ was absorbed maximum absorbance at 228 nm. The resulting linearity overlay spectra is shown in **Figure 2B** and linearity graph was plotted by the absorbance against the concentration of RMZ and the regression coefficient (R^2) was found to be more than 0.999. The results are tabulated in **Table 3**.
In case of HPLC method, the RMZ and ATST peaks were eluted at different time intervals. The working standard solutions of RMZ ranging between 10 μgmL^{-1} to 50 μgmL^{-1} were eluted along with the internal standard ATST. The concentration of the ATST was fixed at 30 μgmL^{-1}. The linearity graph was plotted by taking the values of peak area ratio of RMZ to ATST. With the resulting straight line obtained from the linearity graph as shown in **Figure 3A** and **Figure 3B**, we could validate the precision of the analyst by this method. The regression coefficient (R^2) value was found to be more than 0.999, following the equation Y = MX + C. The results are tabulated in **Table 3**.

Recovery
This parameter shows that the study of accuracy estimation accomplished by the standard solution of the lower, middle, upper and blank, spiked at 60, 80 and 120% against 100%. The results were calculated with the standard procedures and the recovery data were found satisfactorily. The values are shown in **Table 4**. The accepted limits of recovery are in the range of 98-102%. All the observed outcomes are within the range. Hence the proposed method can be adopted in industry unit as well as in educational labs for the assay of RMZ.
The recovery parameter in spectroscopic method was performed by the standard solution of the lower, middle, upper and blank, spiked at 60, 80 and 120% against 100%. The outcomes are found to be satisfactory. The results are tabulated in **Table 4**.

Precision
The precision results of the developed methods were found to be good and in compliance with ICH guidelines. Based on the results of the precision parameter, the HPLC method was found to be precise. The results are revealed in **Table 4**. The repeatability testing was done by six individual spikes. The outcomes of inter-day and intra-day analysis revealed that there was not much deviation in the results and the percentage relative standard deviation (% RSD) was found to be less than 2.0%. Therefore the system suitability of the proposed HPLC method was very good and thereby precision of the system. The results are shown in **Table 4**.
In the spectroscopic method, intra-day and inter-day precision was studied by estimating the consistent responses at three different time intervals on the same day and on three different days by taking different working standard solutions. The percentage of RSD was found to be less than 2.0% i.e., 0.96 and 0.85 for the intraday and inter-day, respectively, which indicate good reproducibility. These results indicated that the precision of the UV spectroscopic method is good. The results are tabulated in **Table 4**.

Robustness studies
The robustness of the HPLC method was studied by a slight deviation in the boosted conditions of the method by injecting the solution of known concentration. The distinctive conditions correspond to variation of flow rate in the mobile phase ranging from 0.9 $mLmin^{-1}$ to 1.1 $mLmin^{-1}$ and change the column oven temperature at 25 ^0C and 30 ^0C.The results are tabulated in **Table 5** which revealed that robustness values are satisfactory and there was no much variance in results and therefore the projected method can be utilized under different conditions. However, in the case of UV spectroscopic method, the robustness parameter was performed by slight modification in detection wavelength by ± 2 nm and outcomes were found to be satisfactory.

Ruggedness

In the ruggedness parameter, standard working solutions were examined by the same chromatographic system on different days using the same column. It was observed from the results that there was a small variation in the peak area and there were no large differences in the retention times. The percentage of RSD was found to be less than 2.0% for RMZ. The resulting data revealed that the developed method is rugged. In the alternate days, the same detector responses were observed and successfully found that the projected method is capable to make results with great precision on different days. Also, ruggedness was determined by using different HPLC instruments by injecting the known concentration of a solution. The detector response, good reproducibility and no variations in retention time indicated that the method is fundamentally rugged. In the spectroscopic method, the ruggedness parameter was examined by using different concentrations of the solution and a slight change in the wavelength. The percentage of RSD did not diverge much in the absorbance value. Hence, the developed method was rugged and can be adopted for the assay of RMZ.

Specificity

Assay

This parameter was carried out by successive separation of RMZ and ATST which was established against placebo which contains potential excipients. In the assay parameter, no interferences were found and both peaks were sharp and separated at the baseline. It was found that no interference of the excipients in the test solution. Therefore the projected HPLC method was established specifically. The obtained results were found satisfactory and are shown in **Table 6**.

In the case of the spectroscopic method, no interferences were found by the placebo of tablet formulations. Therefore the obtained results were acceptable and the results are shown in **Table 6**.

DISCUSSION

Most of the diabetic drug formulations contains pro-drug of gliflozin derivatives like RMZ to prevent diabetic disorder. Several formulations of the diabetic drug contain gliflozin derivative drugs. The literature survey revealed that few analytical methods were developed and validated for the determination of RMZ, *viz*., UPLC, HPLC and UV spectroscopic methods. But most of the methods have one or the other drawbacks. For example, the UPLC is very expensive and hence small scale industries and laboratories cannot afford. Some HPLC and UV spectroscopic methods were developed for the determination of pro-drugs of gliflozin, but not included RMZ. Based on the thorough statistical data analysis (**Table 1)** of the reported methods, it was planned to develop and validated HPLC and UV spectroscopic methods for the assay of RMZ. These analytical methods are simple, sensitive, rapid, rugged, used inexpensive chemicals, involves small sample volume and showed good recovery. The results of all the parameters comply with ICH guidelines.

CONCLUSION

Few RP-HPLC methods were developed for the determination of gliflozin derivatives such as canagliflozin, empagliflozin, metformin hydrochloride. These methods were carried out for the determination of either one or two of the above-mentioned drugs or single drug along with other combination. The projected methods are distinctive from reported methods. In RP-HPLC method, the total run time was 10 minutes. The linearity range for RP-HPLC method was found to be from 10 μgmL^{-1} to 50 μgmL^{-1} and for the UV spectroscopic method it was found to be in the range of 100 to 250 μgmL^{-1}. The values of regression coefficients (R^2) were found to be more than 0.999 for both the techniques. The LOD and LOQ values for UV and HPLC methods were found to be 10.0 μgmL^{-1} and 1.0 μgmL^{-1} and 40 μgmL^{-1} and 3.5 μgmL^{-1}, respectively. The developed methods were found to be simple, accurate, reproducible and precise. The obtained data of both the methods were clearly showed that RP-HPLC method was relatively more sensitive than the UV spectroscopic method.

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Ref. No.	Analytical Method	Drug(s) analyzed	Result(s)	Remarks
1	UPLC/PDA	Simultaneous determination of RMZ and Metformin hydrochloride	Linearity range: 10-100 $ngmL^{-1}$ LOD: 5 and 10 $ngmL^{-1}$ LOQ: 10 and 50 $ngmL^{-1}$	UPLC is very expensive; Small scale industries and laboratories cannot afford.

3	UV-Spectrophotometric	Simultaneous determination of Emphagifolizine and Metformin hydrochloride	Linearity range: 5-25 and 2-12 µg ml^{-1} LOD: Not available LOQ: Not available	Glifolizine pro-drug used for determination with Metformin. Not included RMZ
4	Liquid chromatography	Simultaneous determination of RMZ and metformin hydrochloride	Linearity range: 1–20 µgmL^{-1} LOD: 0.180 µgmL^{-1} LOQ: 0.560µgmL^{-1}	Narrow linearity range.
5	RP-HPLC	Simultaneous determination of Dapagliflozin and Saxagliptin	Linearity range: 20-70 and 20-70 LOD: 0.109 and 0.58 µgmL^{-1} LOQ: 0.332 and 1.77 µgmL^{-1}	Glifolizine pro-drug used for determination. Not included RMZ
6	HPLC	Simultaneous determination of Saxagliptin and Metformin	Linearity range: 5.00-125.00 and 2.50-62.50 µg ml^{-1} LOD: 0.45 and 0.19 µgmL^{-1} LOQ: 1.50 and 0.66 µgmL^{-1}	Other than RMZ drug determined Not included RMZ
7	RP-HPLC	Metformin Hydrochloride and Sitagliptin Phosphate	Linearity range: 10-50 and 20–100 µgmL^{-1} LOD: 0.016 and 0.14 µgmL^{-1} LOQ: 0.048 and 0.42 µgmL^{-1}	Other than RMZ drug determined
8	RP-HPLC	Metformin hydrochloride and Canagliflozin	Linearity range: 25-150 and 2.5-15 µgmL^{-1} LOD: 0.17 and 0.50 µgmL^{-1} LOQ: 0.01 and 0.50 µgmL^{-1}	Other than RMZ drug determined
9	RP-HPLC	Metformin hydrochloride and Canagliflozin	Linearity range: 25-150 and 2.5-15 µgmL^{-1} LOD: 0.134 and 0.124 µgmL^{-1} LOQ: 0.406 and 0.376 µgmL^{-1}	Other than RMZ drug determined
10	Spectrophotometric and Chemometric methods	Empagliflozin and Metformin	Linearity range: LOD: 0.20 and 0.19 µgmL^{-1} LOQ: 0.59 and 0.58 µgmL^{-1}	Other than RMZ drug determined
11	UV Derivative Spectrophotometric Methods	Metformin and RMZ	Linearity range: 1–20 and 2.5–35 µgmL^{-1} LOD: 0.180 and 0.660 µgmL^{-1} LOQ: 0.560 and 1.850 µgmL^{-1}	Derivative method. Narrow linearity range
Proposed methods	RP-HPLC and UV spectroscopic	Remogliflozin Etabonate	HPLC method Linearity range: 10-50 µgmL^{-1} LOD: 1.00 µgmL^{-1} LOQ: 3.50 µgmL^{-1} UV Spectroscopic method Linearity range :100-250 µgmL^{-1}	Employed internal standard. Simple, sensitive and rugged.

			LOD: 10.00 μgmL^{-1} LOQ: 40.00 μgmL^{-1} R^2: 0.999	

[redacted]

Parameter	RMZ	ATST	Limit
Number of theoretical plates	6269	6465	NLT* 2000
Retention time (t_R) in min	6.10	7.00	-
Resolution	-	2.90	NLT* 2.0
Peak asymmetry (A_S)	1.06	1.09	NMT** 2.0
% RSD[#]	0.74	0.82	NMT** 2.0

*NLT – Not less than
**NMT – Not more than
[#] Average of 6 injections

Parameter	RP-HPLC	UV-Spectroscopy
Linear dynamic range (μgmL^{-1})	10-50	100-250
Regression equation (Y^{a})	-	-
Slope (b)	0.028	0.003
Intercept (c)	-0.015	-0.131
Correlation coefficient (r)	0.999	0.999
LOD (μgmL^{-1})	1.00	10.00
LOQ (μgmL^{-1})	3.50	40.00
% RSD*	0.24	0.92

Y^{a} = bX + c, where X is concentration of drug in μgmL^{-1}
* Average of 6 injections and/or scans

Concentration	Amount of drug taken	RP-HPLC				UV Spectroscopy		Limit
		RMZ	%RSD*	ATST	%RSD*	RMZ	%RSD**	
60%	60 $mgmL^{-1}$	99.50	0.95	101.00	0.60	98.50	0.85	98-102%
80%	80 $mgmL^{-1}$	99.00	0.82	99.00	0.65	98.80	0.92	98-102%
120%	120 $mgmL^{-1}$	98.50	0.75	99.50	0.74	99.50	0.97	98-102%
Intra day			0.65		0.72		0.96	NMT-2.0
Inter day			0.59		0.65		0.85	NMT-2.0

RSD: Relative Standard deviation
* Average of 6 injections
** Average of 6 scans

Parameter	Variations	RMZ Retention time	ATST Retention time
Flow rate	1.9 $mLmin^{-1}$	6.45	7.32
	2.0 $mLmin^{-1}$	6.10	7.00
	2.1 $mLmin^{-1}$	5.85	6.64
Temperature	25 ^{0}C	6.10	7.10
	30 ^{0} C	6.20	7.20

Name of the drug	Instruments	Label claims of market sample in mg per tablet	Obtained result in mg per tablet	Assay values (%)	Limit
RMZ	HPLC	200	199	99.5	98.00-102%
	UV spectroscopy	200	197	98.5	98.00-102%

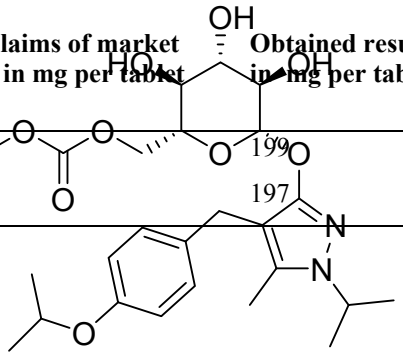
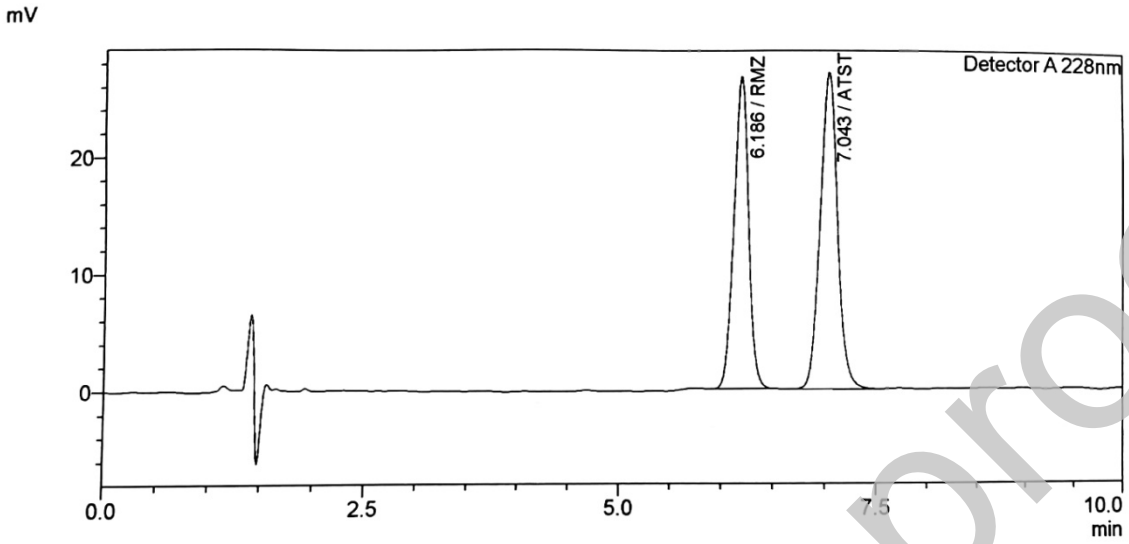


Figure 1. Chemical structure of RMZ



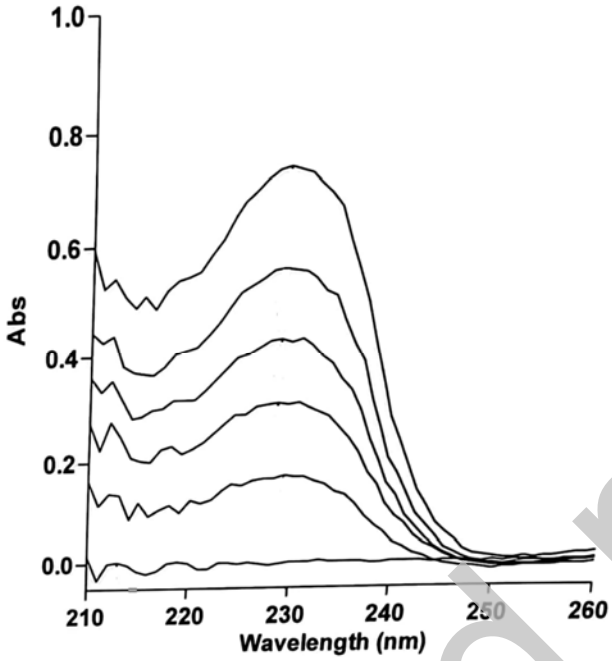
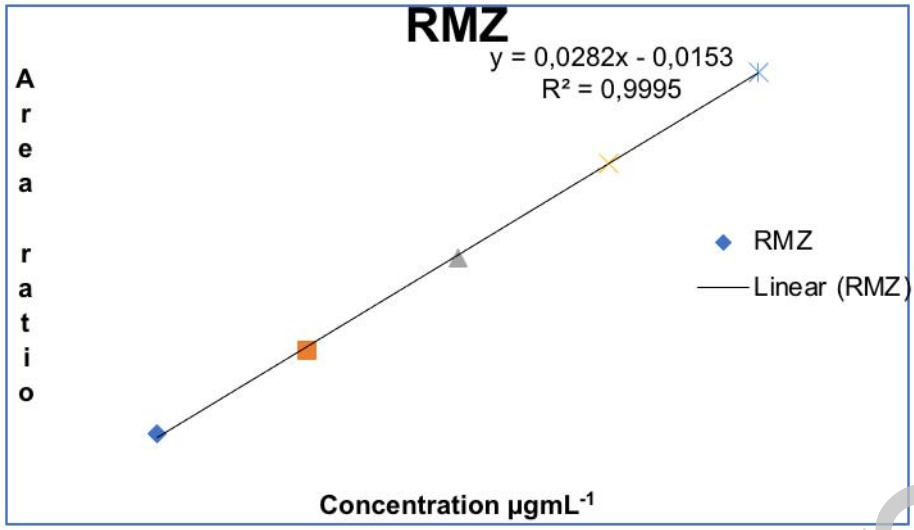


Figure2B. Overlapped linearity graphs of UV-spectroscopic method

(A)



(B)

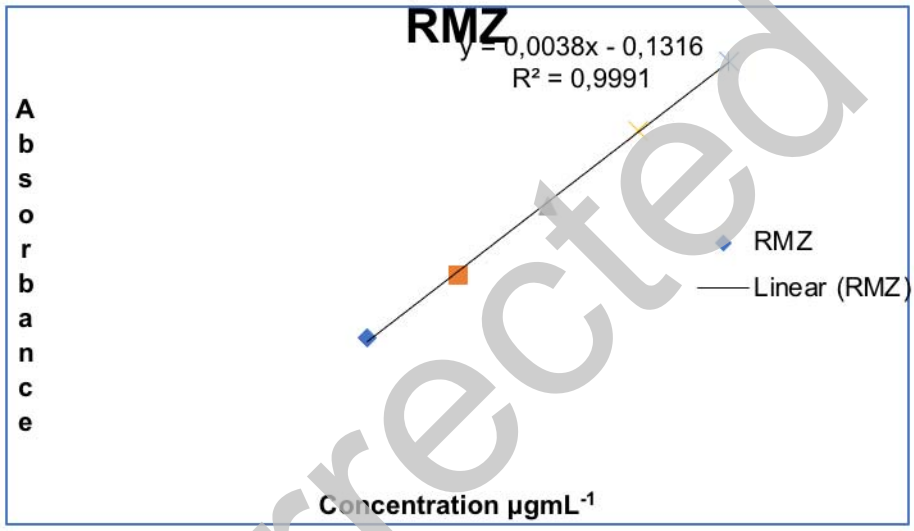


Figure 3. Linearity graphs plotted by RP-HPLC data **(A)** and UV-Visible spectroscopy data **(B)**