GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

RP-HPLC method for estimation of miglitol and metformin hydrochloride in pharmaceutical formulation

Bhanu Teja Reddy Thumkuntla * and Manjunath Sooganna Yalagatti

Department of Pharmaceutical Analysis, Srikrupa Institute of Pharmaceutical Sciences, Siddipet-502 277 (TS), India.

GSC Biological and Pharmaceutical Sciences, 2023, 23(03), 138–146

Publication history: Received on 24 April 2023; revised on 08 June 2023; accepted on 10 June 2023

Article DOI: https://doi.org/10.30574/gscbps.2023.23.3.0219

Abstract

A simple, rapid and sensitive RP-HPLC method was developed for the quantitative determination of metformin hydrochloride and miglitol in combined tablet dosage form. The chromatographic analysis was carried out on Phenomenex Gemini C18 (250 x 4.6 mm, 5 µm) with mobile phase containing methanol: TEA buffer (50:50 v/v). The flow rate of mobile phase was 1.0 mL/min and effluents were monitored at 272 nm. The retention times of miglitol and metformin hydrochloride were 2.121 min and 3.643 min, respectively. The proposed method was validated with respect to linearity, accuracy, precision, specificity and robustness.The method was found to simple, rapid and sensitive and was successfully applied to the estimation of metformin hydrochloride and miglitol in combined dosage form.

Keywords: RP-HPLC; Metformin hydrochloride; Miglitol; Dosage form

1. Introduction

Metformin HCl [figure1] is an oral antidiabetic drug in the biguanide class. It is most widely prescribed antidiabetic drug in the world used to treat type 2 diabetes. Metformin(MET) helps to control the amount of glucose (sugar) in blood. It decreases the amount of glucose and also increases body's response to insulin, a natural substance that controls the amount of glucose in the blood. It is not used to treat type 1 diabetes. It is also used for treatment of gestational diabetes, polycystic ovary syndrome (PCOS). It works by decreasing hyperglycemia primarily by suppressing glucose production by the liver (hepatic gluconeogenesis). It helps to reduce LDL cholesterol and triglyceride levels, and is not associated with weight gain. Metformin comes as a liquid, as a tablet, and as an extended-release (long-acting) tablet taken orally. It is used alone or with other medications . Very rare but serious side effect with Metformin is lactic acidosis. Other than that common side effect are gastrointestinal irritations, including diarrhea, cramps, nausea, vomiting and increased flatulence. Literature survey revealed The HPLC methods for estimation of metformin in Bulk, human plasma and pharmaceutical dosage forms [1–5]. LC-MS-MS method was reported for the determination of Metformin in human plasma [6]. Literature survey reveals several Analytical and Bioanalytical methods for the analysis of Metformin. These methods reported with Metformin alone or in combination with other drug. These include, HPLC [7-11] and spectrophotometric analysis of Metformin in tablets [12-13].

Miglitol [figure2] belongs to a class of drug called alpha-glucosidase inhibitors used to control blood glucose (sugar) levels in type 2 diabetes (non-insulin-dependent diabetes). It is approved by FDA in December 1996. Miglitol(MIG) inhibits glycoside hydrolase enzymes called alpha-glucosidases thereby slowing the appearance of sugar in the blood after meal. It works by slowing down the absorption of carbohydrates from diet, so that blood sugar does not rise as much after meal. Alpha-glucosidase inhibitors are used to help control blood sugar levels that are not controlled by diet and exercise alone. It is believed that strict control of blood sugar in people with diabetes decreases the risk of eye, kidney and nerve damage. Controlling high blood sugar helps to decreases the risk of eye, kidney, nerve damage, loss of limbs and sexual function problems. It is used alone or in combination with a sulfonylurea such as glyburide (Diabeta).

* Corresponding author: Bhanu Teja Reddy T

It is an oral administrative drug available in form of tablet. The most common side effects of miglitol are Gastrointestinal symptoms such as abdominal pain, diarrhea, flatulence and skin rash. Rare but possible side effects include low serum iron. Literature survey revealed that several Analytical and Bioanalytical methods for its estimation using Reversed Phase-High Performance Liquid Chromatography [RP-HPLC] with UV detection, HPLC - electrospray tandem mass spectrometry, LC-MS, liquid chromatography with atmospheric pressure chemical ionization tandem mass spectrometry[14] and RP-HPLC method[15-16]. The developed method has various advantages over the above mentioned methods, as it is simple, economical, faster, precise, accurate and specific for quantitative determination of Miglitol in pharmaceutical dosage form. As per our detailed literature survey as on date, there are very few reports using UV[17] & RP-HPLC[18] for the simultaneous quantitative estimation of Metformin and Migllitol in Bulk & Pharmaceutical dosage forms. The RP-HPLC[19-24] methods have superiority over the UV spectrophotometric methods[25-28] in terms of selectivity and sensitivity. We here in reported a simple, sensitive, precise, accurate, linear and isocratic RP-HPLC method for the simultaneous quantitative estimation of Metformin and Migllitol in bulk & Formulation as per ICH Guilences.

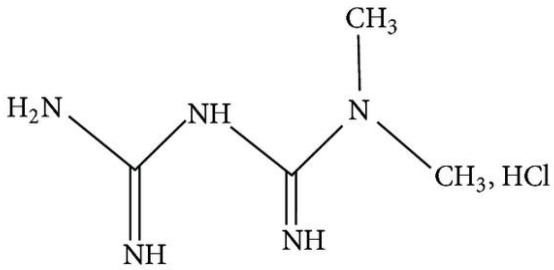

Figure 1 Structure of metformin hydrochloride

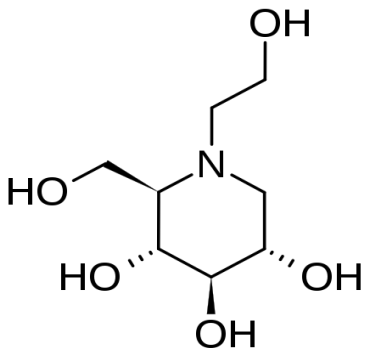

Figure 2 Structure of miglitol

2. Materials and method

2.1. Instrumentation

The technique was developed using an Acquity HPLC system (Waters, Milford, MA, USA) with a model 2996 PDA detector. A Rheodyne micro-litre syringe fitted with a 20 µl loop was used for injection of sample into the column and data were recorded evaluated by use of empower software.

2.2. Materials

Metformin and Migllitol pure samples were procured as gift samples. MIGNAR MF tablets were procured from the local market. Label claim of MIGNAR MF tablets for MET and MIG were 500 mg and 50 mg respectively. Methanol, acetonitrile and glacial acetic acid and water of HPLC grade were purchased from E. Merck and used throughout the experiment.

2.3. Mobile phase preparation

Triethyl amine buffer and methanol in the ratio of 35:65 v/v was used as mobile phase. The mobile phase was sonicated for 15 min in an ultrasonic bath and filtered through nylon membrane disc filter of 0.45 µm pore size using a vacuum pump before pumping into the HPLC system. .

2.4. Preparation of stock standard solution

Metformin hydrochloride (500 mg) and miglitol (50 mg) were accurately weighed and transferred into 100 ml volumetric flask separately. They were dissolved in 100 ml methanol to obtain 5000 µg/ml and 500 µg/ml concentration of stock solutions respectively. From these stock solution 1 ml each of MET and MIG were taken into 10 ml volumetric flasks separately and further diluted with a mobile phase to get 500 µg/ml and 50 µg/ml concentrations of MET and MIG respectively. The solutions were then filtered through 0.45 µm Nylon filter.

2.5. Preparation of sample solution

For analysis of drugs, 20 tablets were weighed and triturated in glass mortar and quantity of powder equivalent to 500 mg of metformin hydrochloride was transferred to 100 ml volumetric flask and dissolved in sufficient quantity of methanol. It was sonicated for 10 min and volume was made up to 100 ml to obtain a stock solution of 5000 µg/ml of metformin hydrochloride and 500 µg/ml of miglitol. This solution was then filtered through nylon 0.45 µm membrane filter. The solution was further diluted with mobile phase to get a concentration of 100 µg/ml and 10 µg/ml of metformin hydrochloride and miglitol respectively. This solution was injected 6 times in to the column and chromatograms were recorded and respective peak areas were measured. The contents of MET and MIG were calculated by using the regression.

2.6. Chromatographic conditions

- Column: Phenomimex Gemini C18 (250 × 4.6 mm, 5 µm particle size)
- Flow rate: 1 ml/min.
- Detection wavelength: 272 nm
- Injection volume: 20 µl.
- Column temperature Ambient.
- Run Time: 10 min.
- Mobile Phase: Methanol : TEA (65 : 35 v/v)
- Run Mode: Isocratic

3. Results And Discussions

3.1. Optimization of chromatographic conditions

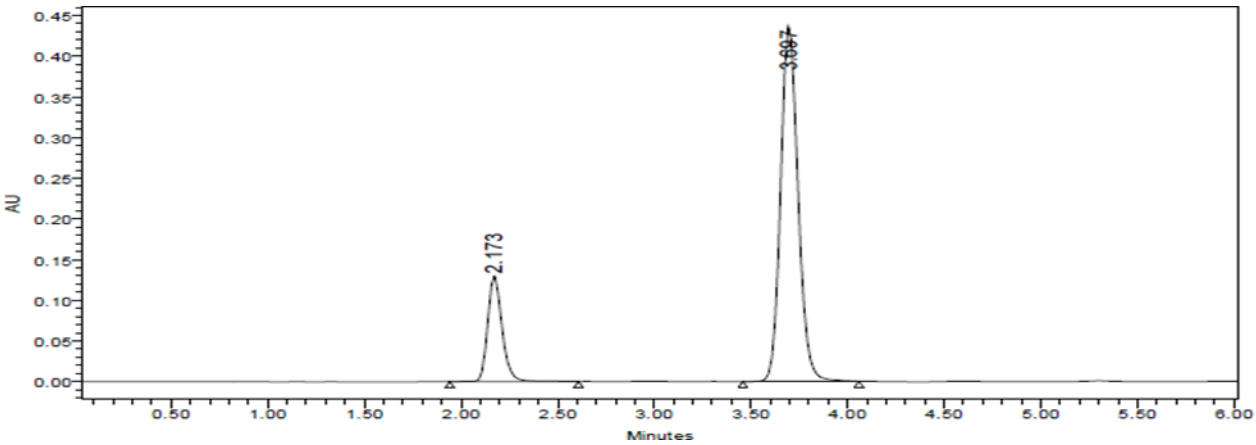

Figure 3 Chromatogram of miglitol and metformin hydrochloride

Several HPLC methods were reported for the estimation of metformin hydrochloride and miglitol using methanol, water, acetonitrile and phosphate buffer as mobile phases.With a intention to develop a HPLC method with less

retention time for MET and MIG we tried with methanol and TEA buffer using Phenomimex Gemini C18 (250 × 4.6 mm i.d., 5µ) column. Different trails were performed using different proportions of methanol and TEA buffer. The mobile phase containing methanol and TEA buffer in the composition of 65:35 v/v was found to be satisfactory and gave symmetric and well resolved peak for MET and MIG. The retention time of miglitol and metformin hydrochloride was found to be 2.152 min and 3.646 min. The standard chromatogram was shown in Figure 3.

3.2. Method validation

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines [29].

3.2.1. System suitability

System suitability is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. % RSD on five replicate injections of standards solution was calculated. The results of system suitability for MET and MIG are shown in Table 1.

Table 1 Chromatographic characteristics of system suitability

PARAMETERS	VALUE (Mean* ± SD)	
	MET	MIG
Peak Area	1657592±0.08	405128±0.003
Tailing Factor	1.25±0.04	1.12±0.01
Theoretical Plate	4373 ±0.06	6738 ±0.02
HETP	36.3±0.01	38.7±0.03
Retention time	3.654 min	2.172 min

*mean of six determinations

3.2.2. Linearity

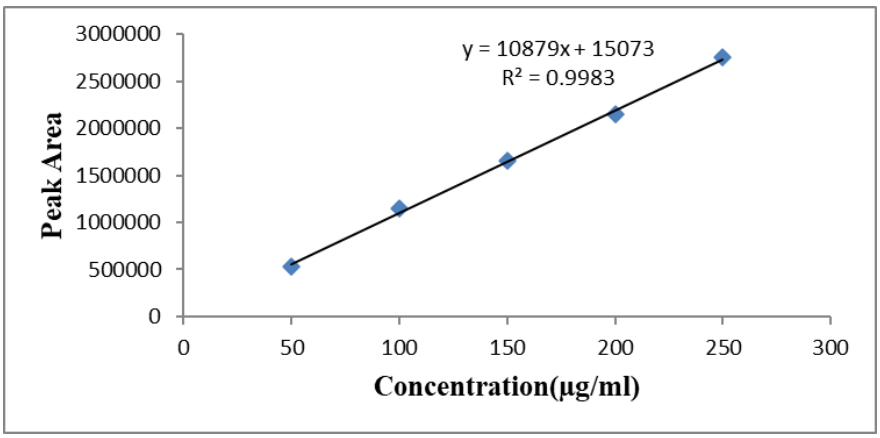

Figure 4 Calibration curve for metformin hydrochloride

Calibration graphs were constructed by plotting peak area vs concentration for metformin hydrochloride and miglitol. The calibration graphs were plotted concentrations in the range of 50-250 µg/ml and 5–25 µg/ml for metformin and miglitol respectively. The regression line obtained was linear. From the data obtained, co-relation coefficient, slope and y-intercept were calculated. The linearity of the method was excellent as evidenced by the correlation coefficient of 0.999 for both drugs. The regression equation and other parameters obtained from the plot are mentioned in Table 1.

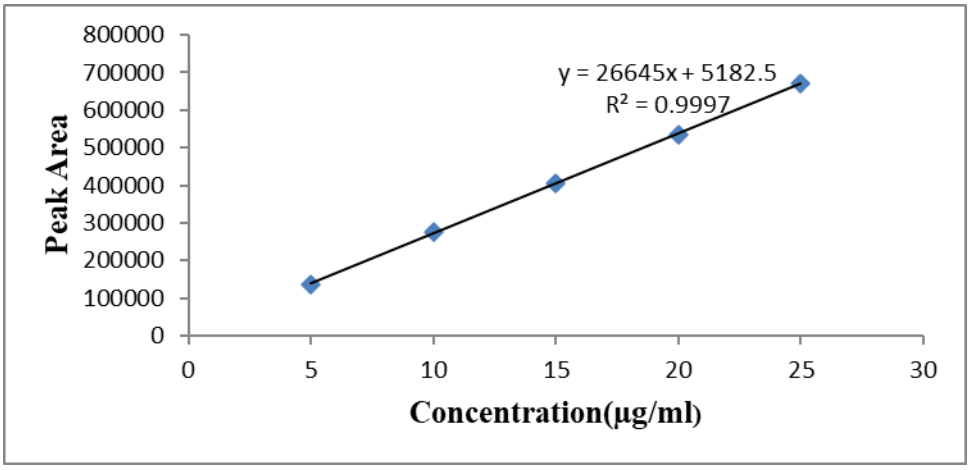

Figure 5 Calibration curve for miglitol

Table 2 Linear regression analysis data

Parameter	MET	MIG
Linearity	50 – 250 µg/ml	5-25 µg/ml
Regression Equation	Y= 10879x+15073	Y= 26645+5182.5
Correlation Coefficient	0.9983	0.9997

3.2.3. Precision

Precision was evaluated by injecting six replicate injections of metformin hydrochloride and miglitol of sample solution under the same chromatographic conditions and calculated by the % RSD. The intraday and interday precision study were conducted for both metformin HCl and miglitol. The % RSD indicates that the developed method is repeatable. The % RSD for assay of metformin hydrochloride and miglitol was found to be 1.72 and 0.36. The results are shown in Table 3. Both inter-day and intra-day R.S.D. were less than 2 %, indicating a sufficient precision of the developed method.

Table 3 Precision studies of metformin hydrochloride and miglitol

Amount of std taken (µg/ml)	Intra-day Precession (n=6)		Inter-day Precession (n=6)	
	Mean±SD	%RSD	Mean ± SD	%RSD
Metformin Hydrochloride	119398.16±1795.87	1.59	119389±1224.69	0.88
	1657592.23±2690.77	1.18	1657577.3±3461.82	1.46
	2150492.24±3132.79	0.90	2150489.5±3040.94	0.83
Miglitol	277123.17±27786.33	1.05	277176.6±23880.28	1.10
	405128±30278.66	0.46	404823.9±27079.26	0.41
	564653.5±16657.17	0.17	564686.3±14805.89	0.15

3.2.4. Accuracy

In order to judge the quality and applicability of method the recovery analysis was performed at three levels 50 %, 100 %, and 150 % by standard addition method. The % recoveries for Metformin Hydrochloride and Miglitol were calculated and it was found to be within the limits; the results are given in Table 4.

Table 4 Accuracy Data

Analyte	% Level	Nominal value (mg)	Found (mg)	Mean% Recovery	%RSD
Miglitol	50	25	25.01	100.06	0.05
	100	50	50.09	100.14	0.20
	150	75	75.30	100.4	0.37
Metformin Hydrochloride	50	2.5	2.48	99.34	0.21
	100	5	4.91	98.37	0.36
	150	7.5	7.47	99.66	0.17

3.2.5. Specificity

Since bulk and tablet formulations are made of different components and excipients, the specificity was carried out through the comparison of the peak retention time of the formulations with paracetamol and tramadol hydrochloride standard drug sample and blank solution. No interference of the excipients was detected since no peak was detected in the same retention time of miglitol and metformin hydrochloride.

3.2.6. Robustness

The robustness as a measure of method capability to remain unaffected by small, but deliberate changes in chromatographic conditions was studied by testing influence of small changes in mobile phase composition (10 % absolute change in organic phase) and flow rate (±0.2 mL/min) and wavelength (± 2 nm). The theoretical plate count and tailing were within the limits. So, the method was found to be robust with respect to variability in all robust conditions.The results are shown in Table 5 and Table 6.

Table 5 Robustness results for miglitol

Conditions	% Assay	System Suitability parameters	
		Theoretical Plates	Tailing Factor
Flow Rate 0.8 mL/min	99.68	6792	1.08
Flow Rate 1.2 mL/min	99.49	6747	1.12
Mobile Phase- Methanol(70): TEA (30)	99.67	6762	1.12
Mobile Phase- Methanol(60): TEA(40)	99.84	6649	1.11
Wavelength 270 nm	99.83	6839	1.12
Wavelength 274 nm	99.61	6869	1.12

Table 6 Robustness results for tramadol hydrochloride

Conditions	% Assay	System Suitability parameters	
		Theoretical Plates	Tailing Factor
Flow Rate 0.8 mL/min	99.55	4665	1.21
Flow Rate 1.2 mL/min	99.68	4217	1.26
Mobile Phase- Methanol(70): TEA (30)	99.83	4363	1.24
Mobile Phase- Methanol(60): TEA(40)	99.63	4261	1.23
Wavelength 270 nm	99.87	4107	1.23
Wavelength 274 nm	99.69	4316	1.24

3.2.7. LOD and LOQ

The LOD and LOQ of tramadol hydrochloride and paracetamol were determined by using the signal to noise approach as defined in ICH guidelines. The results are given in Table 7.

Table 7 LOD and LOQ

Drug	LOD (µg/ml)	LOQ (µg/ml)
Metformin hydrochloride	0.15	0.45
Miglitol	0.04	0.12

3.2.8. Ruggedness

Ruggedness of the developed method was determined by analyzing six sample solutions of by two analysts in the same laboratory to check the reproducibility of the test result. The % recovery and standard deviation were calculated in both cases.The result was shown in Table 8.

Table 8 Ruggedness

Sample	Analyst - 1	Analyst-2
	(Mean±%RSD)	(Mean±%RSD)
Metformin HCl	1657592±1.05	1657889.7±0.96
Miglitol	405182±0.41	405192±0.19

3.2.9. Assay of pharmaceutical formulation

The proposed validated method was successfully applied to determine metformin hydrochloride and miglitol in its tablet dosage form. The result obtained for metformin hydrochloride and miglitol was comparable with the corresponding labelled amounts and they are given in Table 9.

Table 9 Analysis of miglitol and metformin HCl in commercial formulation

Formulation	Labelled claim(mg)		Amount found*(mg)		%Recovery*±%RSD	
	MET	MIG	MET	MIG	MET	MIG
MIGNAR MF Tablets	500	50	498.65	49.66	99.73±0.34	99.33±0.26

*Average of three determinations

4. Conclusion

The present work refers to the fact that the most accurate, precise, and robust HPLC method was developed and validated for estimation of metformin hydrochloride and miglitol in pharmaceutical dosage form in accordance with the ICH parameters. The method was validated and found to be simple, rapid, accurate, and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore, the proposed method can be used for routine analysis of metformin hydrochloride and miglitol in its dosage form.

Compliance with ethical standards

Acknowledgments

The authors are grateful to management of Srikrupa Institute of Pharmaceutical Sciences for providing necessary facilities to carry out the above research work.

Disclosure of conflict of interest

No conflict of interest.

References

[1] Arayne MS, Sultana N, Zuberi MH. Development and validation of RP-HPLC method for the analysis of metformin. Pakistan journal of pharmaceutical sciences. 2006;19(3):231-5.

[2] Madhukar A, Prince A, Vijaykumar R, Sanjeev Y, Raghuprata D, Jagadeeswar.K. Simple and Sensitive analytical method development and validation of Metformin hydrochloride by RP-HPLC. Journal of Pharmacy and Pharmaceutical Sciences. Journal of chromatographic science. 2011;3(3):117-20.

[3] Narendra KT, Mohan RK, Sreenivasulu K, Raju R, Chandra NS. Novel RP-HPLC method for the Estimation of metformin hydrochloride In pharmaceutical dosage forms. International Journal of Science Innovations and Discoveries. Journal of chromatographic science. 2011;1(3):395-421.

[4] Mousumi K, Choudhury PK. HPLC method for estimation of metformin hydrochloride in formulated microspheres and tablet dosage form. Indian J Pharm.Sci . 2009;71(3):318-20.

[5] Florentin T, Monica A. Specificity of an analytical HPLC assay method of metformin hydrochloride. Journal of chromatographic science 2007;52(6):603-9.

[6] Yunbiao W, Fawcettb J. Yingwu Jingkai Gua,, Paul Xu Bai. Rapid and sensitive liquid chromatography tandem mass spectrometric method for the quantitation of metformin in human plasma. Journal of Chromatography B . 2004;808(2):215-9.

[7] Kumar TH , Sankar DG. RP-HPLC method for simultaneous estimation of Metformin Hydrochloride and Canagliflozin in pharmaceutical formulation. Indian Drugs. 2021; 58(6) :49-59.

[8] AbuRuza S, Millershipb BJ, McElnayb J, Chromatography B. The development and validation of liquid chromatography method for the simultaneous determination of metformin and glipizide, gliclazide, glibenclamide or glimperide in plasma. Journal of Chromatography B. 2005;817(2):277-86.

[9] Sultana N, Arayne MS, Shafi N, Siddiqui FA, Hussain A. Development and validation of new assay method for the simultaneous analysis of diltiazem, metformin, pioglitazone and rosiglitazone by RP-HPLC and its applications in pharmaceuticals and human serum. Journal of chromatographic science 2011;49(10):774-9.

[10] Narsimha RD, Chandana M, Hplc, Pioglitazone H. Method Development and Validation of RP-for Simultaneous Analysisof Three Component Tablet Formulation containing Metformin and Glibenclamide. International Journal of PharmTech Research 2012;4:948-56.

[11] Amruta B, Minal R, Ghante SD, V. U. Loni, Sawant . Simultaneous Method for Estimation of Sitagliptin phosphate and Metformin hydrochloride in Bulk and Tablet Dosage Form. Der Pharma Chemica 2012;4(3):854-9.

[12] Arayne MS, Sultana N, Zuberi MH, Siddiqui FA. Spectrophotometric Quantitation of Metformin in Bulk Drug and Pharmaceutical Formulations using Multivariate Technique. Indian journal of pharmaceutical sciences. 2009;71(3):331-5.

[13] Narasimha RD, Prasada RM, Hussain J, Sumanoja S, Rajeswara RV. Lakshmi N. Method development and validation of forced degradation studies of metformin hydrochloride by using uv spectroscopy. IJPCBS 2013;3(3) :546-53.

[14] Li X, Wang Y, Wang J, Fawcett JP, Zhao L, Gu J. Determi nation of miglitol in human plasma by liquid chromatography/tandem mass spectrometry. Rapid communications in mass spectrometry . 2007;21(2):247-51.

[15] Shrivastava B, Baghel US, Sahu M. Stability-Indicating RP-HPLC Method for Estimation of Miglitol in Bulk and Tablets. Indian journal of pharmaceutical sciences. 2010;72(6):781-4.

[16] Chungath T, Padmanabha RY, Devanna N. Development of validated RP-HPLC method for the estimation of Miglitol in tablet formulations. Research Journal of Pharmaceutical Biological and Chemical Sciences 2011;2(3):807-13.

[17] Sejal RP, Prachi VK, Ritu VK, Ravikiran M, Nargun LVG. Development and Validation of Analytical Method for Quantitative Estimation of Miglitol and Metformin in Combined Dosage Form. Journal of Applied Pharmaceutical Science. 2012;2(6):227-9.

[18] Ahmad S , Sajjad A, Rageeb M, Usman M, Imran M, Akhtar R. Development and Validation of RP- HPLC Method for Simultaneous Estimation of Metformin and Miglitol in Bulk and Dosage Form. Asian J. Pharm. Res. 2017; 7(3): 139-147.

[19] Rao MT, Ratna VJ , Rao YS ,Kumar TH.Development and Validation of RP-HPLC Method for the Determination of Itraconazole in Bulk and Capsule Dosage Form. Int. J. Pharm. Sci. Rev. Res.2015; 31 : 221-225.

[20] Sindhu SN , Rao YS ,Kumar HT, Rao KVP.Method development and validation of RP-HPLC method for estimation of imatinib mesylate in pure and pharmaceutical dosage form. 2015; Der Pharmacia Lettre, 7 (3):33-38.

[21] Kumar TH , Sri, DS, Rao, KVP, Rao YS.Validated RP- HPLC Method For Determination Of Rosuvastatin Calcium In Bulk And Pharmaceutical Formulation.2015; IJPSR, 6(7): 1000-05.

[22] Kumar TH, Prajna CKK ,Rao KVP, Rao YS.RP-HPLC Method For Estimation Of Tramadol Hydrochloride And Paracetamol In Pharmaceutical Formulation. GSC Biological and Pharmaceutical Sciences. 2019; 8(1):89-97.

[23] Kumar HT, Asha C, Sankar DG. Estimation Of Amlodipine Besylate And Irbesartan In Pharmaceutical Formulation By RP-HPLC With Forced Degradation Studies. International Journal of Applied Pharmaceutics 2019;11(33):159-167

[24] Kumar HT, Begum A , Sankar DG. RP-HPLC Method For Determination of Azilsartan Medoximil and Chlorthalidone in Tablets exposed to Forced Degradation Condition. Biomedical Research 2019;30(5):775-785.

[25] Kumar HT , Reddy M., Rishika D , Kumar PR. Estimation of Orlistat by UV Spectrophotometric method. IJPSR. 2011; 2 : 2469-2471.

[26] Madhuri PL , Kumar TH, Rao YS , Rao KVP.UV Spectrophotometric Method for Estimation of Sacubitril in Synthetic Mixture. Asian J. Research Chem. 2019; 12 : 07-10.

[27] Chaitanya PSV, Kumar,TH, Rao YS, Rao KVP. Estimation of vortioxeine in bulk and pharmaceutical formulations by UV spectrophotometry. European Journal of Pharmaceutical and Medical Research. 2019; 6 : 389-392.

[28] Sankar DG ,Kumar TH , Krishna MV.UV Spectrophotometric determination of Duloxetine Hydrochloride. Asian Journal of chemistry. 2019, 21(6).

[29] ICH Validation of analytical procedures: text and methodology. In International Conference on Harmonization Q2 (R1). Geneva; 2005.