Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 3, 2014

Original Article

DESIGN AND STUDY OF REVERSE PHASE ULTRA HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR SIMULTANEOUS ESTIMATION OF TWO ANTIDIABETIC DRUGS WITH AN ANTIHYPERLIPEMIC DRUG USING STATISTICAL APPROACH

KARUNANIDHI SANTHANA LAKSHMI AND SEETHARAMAN RATHINAM*

Department of Pharmaceutical Analysis, College of Pharmacy, SRM University, Kattankulathur, Tamilnadu 603203, India.
Email: seerampharm@gmail.com

Received: 19 Feb 2014, Revised and Accepted: 08 Mar 2014

ABSTRACT

Objective: The foremost objective of this paper is to develop and justify a sensitive, novel Ultra High Performance Liquid Chromatographic technique to simultaneously evaluate two antidiabetic drugs namely Metformin Hydrochloride (MFN), Glimepride (GPE) with an anti-hyperlipidemic drug Atorvastatin Calcium (ATR).

Methods: The technique was built utilizing thermo C_{18} (4.6 mm x 50 mm, 1.9 µm) column having a mobile phase comprising of 10mM ammonium dihydrogen phosphate pH regulated to 3.00 with weakened Orthophosphoric acid as buffer, with a ratio of buffer: acetonitrile 50:50 (v/v) and with a surge percentage of 0.3mL min^{-1}. The finding out was carried out at 255 nm utilizing a photo diode range detector.

Results: The retention times for MFN, ATR and GPE were 0.4, 1.3 and 1.6 min respectively. In order to designate suitability in the experimental design approach, a robustness test was carried out. To assess robustness 3 aspects were taken into consideration, namely, proportion of flow rate, proportion of acetonitrile in movable stage and pH; all the three factors have no significant effect on response (assay). On the assay for the simultaneous estimation of MFN, ATR, GPE provided an effective approach by using the robustness test along with full factorial design (FFD).

Conclusion: This method was successfully used to analyze fixed dose tablets samples of MFN, ATR and GPE. The chromatographic separation pertaining to the selected analyses was attained in lesser than 2 minutes. The proposed technique can be utilized for regular lab investigation of MFN, ATR as well as GPE in tablets.

Keywords: UHPLC, Experimental design, Robustness, Motorman Hydrochloride, Atorvastatin calcium, Glimepride.

INTRODUCTION

Type 2 diabetes is categorized with a grouping of marginal insulin resistance and insufficient insulin produced by the pancreas. Insulin resistance that is credited to high quantities of free fatty acids and pro-inflammatory cytokines in the plasma results in reduced glucose transfer to muscle cells, higher hepatic glucose generation, and augmented breaking down of fat [1].

MFN, chemically a biguanide derivative enhances glycemic management by reducing hepatic glucose generation, reduced glucose take-in; Metformin improves hepatic as well as peripheral tissue sensation to insulin in addition to augmented insulin-induced glucose uptake. The method of functioning of glimepride in decreasing blood glucose appear to rely on fuelling the discharge of insulin from working pancreatic beta cells, and insulin sensitivity increase in the peripheral tissues. [2-3].

The binding of GPE to the sensitive potassium channel receptors which is present in the pancreatic cell surface happen which leads to the reduction in potassium conductance causing membrance depolarization. Through voltage sensitive calcium channels, calcium ion influx will be stimulated by the membrane depolarization. This augmentation in intracellular calcium ion strength brings about the production of insulin [4-5].

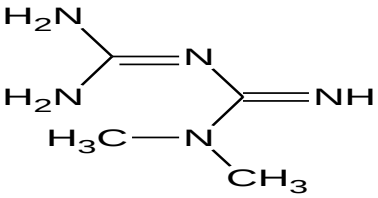
ATR chemically [R-(R*,R*)]-2-(4-fluoro-phenyl)-β, δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenyla-mino)carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate, happens to be a member belonging to the drug class called statins This enzyme acts as a aggressive element that inhibits of hydroxymethylglutral coenzyme (HMG CoA) which is basically an rate-determining enzyme in the biosynthesis of cholesterol through mevalonate passageway. The alteration of HMG-CoA towards mevalonate is increased by HMG-CoA enzyme that is utilized for bringing down cholesterol levels. An increase in Hepatic uptake of cholesterol is realized when the hepatic cholesterol levels are decreased which in turn reduces the levels of plasma cholesterol.

When there is complete or relatively deficient insulin or insulin resistance is experienced due to hyperglycemia and altered lipid, protein and carbohydrate metabolisms, Daibetes Mellitus, a common metabolic disorder occurs which have close association between diabetic dyslipidemia [6-8]. 80 percent of diabetic death happens due to cardiovascular complications. Evidence proves that the dyslipidemia and hyperglycemia are associated with high risk of cardiovascular diseases. Minute solid LDL elements, connected with elevated CHD threat, are highly concentrated in the Diabetic patients. In order to treat diabetic dyslipidemia, LDL level lowering has to be prioritized. When it comes to treating the type 2 diabetes, the agents which we choose must act beyond their blood glucose effect. The Drug therapy must not only impact the blood glucose level, but also have advantageous impact on the obesity, dyslipidemia, hypertension, insulin resistance and, hyperinsulinemia and is likely to be the most useful therapy in the treatment of type-2 diabetes [9-10].

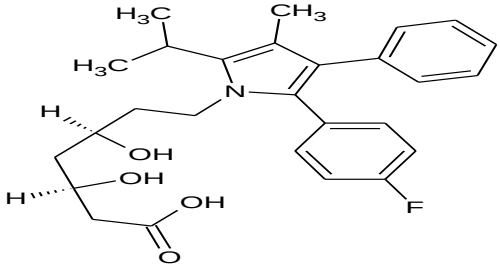
The literature reveals, several Ultra High Performance Liquid Chromatographic (UHPLC) techniques were documented to establish MFN [11 –12], ATR [13 –22] and GPE [23] individually or with some other drugs in pharmaceutical and biological matrixes. To date, no UHPLC technique is reported to concurrently determine MFN, ATR as well as GPE in medicinal dose as tablets.

A significant reduction in division period and solvent utilization is favoured from UHPLC. Research papers reveal that UHPLC structure permits approximately nine times reduction in period for investigation in comparison to the conventional HPLC structure utilizing 5 µm unit dimension analytical columns, and approximately 3 times reduction in investigation period when compared to 3µm unit dimension analytical columns with no concession on the whole division. Investigational method was utilized for the substantiation to assess the strength of the method. The objective of this paper is to address the robustness of UHPLC assay method and to explore the significant factors from a FFD. It also provides an effective case study on the experimental design application on the assay method of a pharmaceutical dosage form.

(a)



(b)



(c)

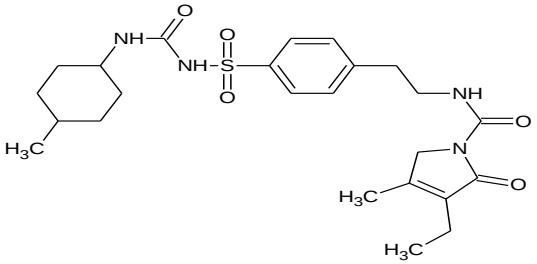


Fig.1: The Chemical structure of a) Metformin Hydrochloride, b) Atorvastatin calcium, c) Glimepride

MATERIALS AND METHODS

Insrumentation and Apparatus

The UHPLC structure utilized for scheme advancement and substantiation happens to be Thermo accela equipped with 1050 quaternary pump auto sampler and a photodiode range detector. The yield of the detector was documented and developed utilizing Chrome quest software version 5.0, Sonicator (PCI bath sonicator) was utilized for degassing of movable stage as well as sonication of the liquids prepared.

Software

The investigational method and data examination were done by utilizing Unscrambler X edition 10.1; other statistical calculation for the analysis was performed by using Microsoft Excel 2007 software (Microsoft, USA).

Chemicals, pharmaceutical preparation and reagents

Reference norms of MFN, ATR calcium as well as GPE were kindly gifted by Ideal Analytical and Research Institution (Puducherry, India) with stated purity of 99.9%, 99.3% and 99.4%, correspondingly. All the values were used as received. Market sample of Statot – GM2 (Abbott Limited, Mumbai, India Batch no.SGM0206) pills were obtained from retail drug store. HPLC quality water, methanol, acetonitrile, analytical reagent category of orthophosphoric acid was obtained from Rankem, India.

Chromatographic conditions

The chromatographic partition was done on a Thermo C_{18} 50 x 2.1, 1.9 µm particle size. Movable stage comprises of mixture of 10mM ammonium dihydrogen phosphate buffer (pH attuned to 3.00 with weakened orthophosphoric acid), acetonitrile to the proportion of 50:50 (v/v). The flow speed and injection quantity was 0.3 mL min^{-1} and 2 µL respectively. The column warmth was ambient and the zeniths were observed at 255 nm.

Preparation of diluent

Diluent 1: Diluent 1 consists of a solution of water, methanol as well as acetonitirile in the percentage of 50:25:25 (v/v/v).

Diluent 2: mobile phase

Preparation of standard solutions

Stock standard mixtures containing MET, ATR plus GPE (5000 µg mL^{-1} of MFN, 100 µg mL^{-1} of ATR, 20 µg mL^{-1} of GPE) were produced by mixing suitable quantities of the compounds in diluent 1. Working mixtures 500 µg mL^{-1} of MFN, 10 µg mL^{-1} of ATR, 2 µg mL^{-1} of GPE were produced from the fore said stock mixture in diluent 2 for test inference.

Preparation of sample solution

20pills of Stator- GM2 were taken; their standard heaviness was established and powdered to a good homogenous dust. An precisely measured amount of the powder corresponding to one tablet (500 mg of MFN and 10 mg of ATR as well as 2mg of GPE) was kept in a 100 mL volumetric flask. To this flask, approximately 70 mL of diluent 1 was included and sonicated for a time of 5 min in a sonicator. With the diluent 1, this mixture was later thinned to the mark and blended thoroughly and strained via a whatmann no. 41 filter paper and the remains was saved following the rejecting the initial small number of millilitres. One millilitre of the residue was poured into a 10 mL volumetric flask, thinned to capacity with mobile phase and blended thoroughly.

Analytical Method validation

System suitability

So as to confirm the system functioning, system appropriateness parameters were measured. With six repeated additions of customary arrangements, system accuracy was decided. Every significant feature together with capability aspect, peak resolution, plus theoretical plate number was calculated.

Specificity

The ability of the technique, to determine the analyte reaction, when there are additional elements like impurities, degradation goods and medium, is called the specificity of an analytical technique. [24]. Based on the sample preparation procedure, an investigative placebo solution (including all the inactive substances other than MFN, ATR as well as GPE) was produced and injected. With the help of this developed method, the interference of these excipients is analyzed for a mixture of inactive ingredients, commercial pharmaceutical preparations including MFN, ATR, and GPE and standard solutions,

Linearity

Linearity was performed between 70% and 130% of normal strength utilizing smallest seven calibration intensities (70%, 80%, 90%, 100%, 110%, 120% and 130%) for all the compounds. The technique of linear regression was utilized to assess the data. The standard compounds' pinnacle region was planned against relevant strengths. Linearity was explained by the formula and associated coefficient was also concluded.

Precision

Precision was examined utilizing the proposed method for six genuine samples of commercial pills (Stator GM 2).

Repeatability

Repeatability or intraday accuracy was assessed by under taking six self-determining evaluations of MFN, ATR and GPE (500µg mL^{-1} of MFN, 10µg mL^{-1} of ATR and 2µg mL^{-1} of GPE) of trial examples against competent reference benchmark on the same day.

Intermediate Precision

Intermediate or Inter-day accuracy was assessed by under taking six self-determining evaluations of MFN, ATR and GPE (50 µg mL^{-1} of MFN, 10µg mL^{-1} of ATR and 2µg mL^{-1} of GPE) of trial examples

against competent reference benchmark by various analysts on various days in the same lab.

Accuracy

With the standard addition method, revival trials were carried out for verifying the correctness of the proposed technique. 80%, 100% and 120% are the three different standard levels added to pre-analyzed pill samples in threes. The proportion of recovery of MFN, ATR and GPE at every stage and every duplicate was established.

The relative standard deviation and the average of percentage recoveries (n = 9) was measured.

Robustness

Selection of factors

The factors assessed are the flow ratio (A), proportion of acetonitrile (B) as well as pH (C). The selected factors are studied at two levels symmetrically situated around the nominal one. Table 1 illustrate the selected factors and the range investigated.

Table 1: Selected factors and range investigated during robustness testing

Factor	Levels		
	(-)	Nominal (0)	(+)
(A) Flow rate (µL min $^{-1}$)	270	300	330
(B) Acetonitrile (%)	48	50	52
(C) pH	2.8	3.0	3.2

Experimental design

As described by the ICH, the strength of an investigative process signifies its ability to stay unchanged by minute and intentional differences in process strictures [25-26]. To evaluate the concurrent difference of the features on the measured reactions, a multi-dimensional technique applying the experimental invent in robustness test is engaged. So as to investigate the concurrent difference of the features on the measured reactions, a multi-dimensional technique utilizing pattern of trials is suggested in robustness assessments.

Factorial Design

Full factorial investigational plan with two or more features wherein all the stages of every feature is connected. It could be further referred to a completely-crossed plan.

A complete factorial research plan permits one to understand the impact of every feature on the reaction variables and the impacts of interactions among features. The quantity of trials to be carried out is a role of the amount of features and the amount of stages for every feature: For instance for k features having 2 levels for every feature, it is possible carry out 2^k trials. A more common method is p^k, wherein p represents the amount of levels and k represent the amount of features examined with p levels. If the amount of levels differs pertaining to its features, the amount of trials is determined by the result of the different levels [27-28]. This type of method is frequently utilized for widespread research of the impacts of a small number of variables, particularly if a number of variables contain different levels that are two or more. They are furthermore suitable as sophisticated screening plans, to investigate key impacts and interactions. A complete factorial plan allows you to investigate the chief impacts and interactions of a small number (2 to 6) of plan variables on one or more reactions. Between two and five core replications are commonly done to ascertain the investigational fault variance and to check the analytical soundness of the method [29-30]. A complete factor factorial plan was used in robustness testing for the selected factors not exceeding three levels (−1, 0, +1); the plan employed in robustness tests of MFN, ATR as well as GPE was a full factorial plan. The investigational domains of the particular variables plus the equivalent reactions are documented in Table 7.

Every one of the trials was carried out in a arbitrary manner to reduce the impacts of unrestrained variables which might bring in a prejudice on the dimensions. Three duplicates of the core features were carried out to assess the investigational fault. The notation for a linear regression method containing three predictor variables with interactions is

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3 + \varepsilon \quad \text{Equation -1}$$

Wherein Y is the reaction of the model, β is the regression coefficient and X_1, X_2 and X_3 symbolize features A, B and C correspondingly, β_1, β_2 and $\beta 3$ are the impact coefficients for the main effects of factors A, B as well as C, correspondingly. β_{12},β_{13} and β_{23} are the impact coefficients for the AB,AC as well as BC interactions, whereas β_{123} symbolizes the ABC interface.

The equation for the regression method is very suitable, particularly if there is a huge amount of higher order interactions existing.

By utilizing the method, a reaction surface regression investigation for the comeback of MFN, ATR as well as GPE was performed by using Unscrambler X 10.1 software.

RESULTS AND DISCUSSION

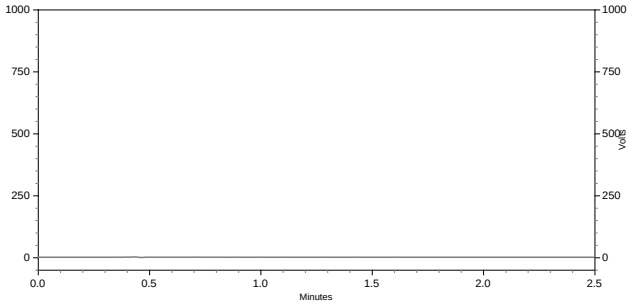
The formatted chromatographic model was authenticated for structure appropriateness, specificity, linearity, array, accuracy, precision and strength as per ICH norms [25].

Specificity

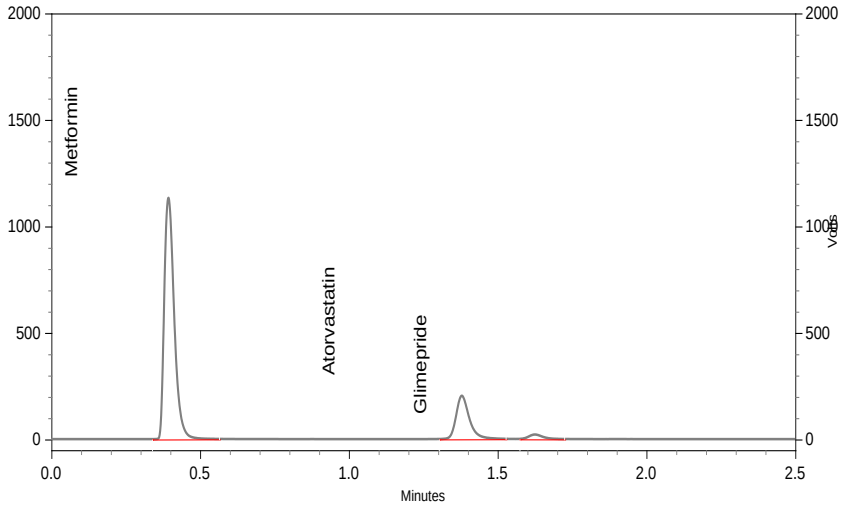
The chromatogram in Fig.2a shows that there is no peak at the retention period of MFN, ATR as well as GPE which indicates the specificity of the proposed model.

System suitability

The percentage R.S.D. of retention period plus peak region of MFN, ATR and GPE of six duplicate injections of standard solution was lower than 2.0%. The findings of structure accuracy are illustrated in Table 3. The % R.S.D values were for duplicate injections which that the structure is accurate. Findings of other system appropriateness strictures like capacity feature, resolution as well as hypothetical plates are illustrated in Table 2 and were within the specified limits. The chromatogram of mixed standard of MFN, ATR and GPE is shown in Figure 2.



(2a)



(2b)

Fig.2: (a) Chromatogram of blank (b) Chromatogram of mixed standard solution MFN, ATR and GPE.

Precision

The standard % evaluation (n = 6) of MFN, ATR and GPE were 99.55%, 99.74 % and 99.65% having %R.S.D. of 0.62%, 0.22% and 0.18%, correspondingly. Findings are illustrated in Table 3 together with intermediary accuracy statistics. Lower values of R.S.D., points that the model is accurate.

Linearity

The reaction was established linear between 70% and 130% of normal strength. For every compound the connected coefficient was more than 0.9990. Correlation coefficients and linearity formulas are illustrated in Table 4. The results indicate very good linearity.

Accuracy

The quantity claimed was contained by ± 3% of quantity included that showed that the technique is precise and in addition exclude the intrusion owing to excipients existing in pills. The Table 5 reveals the findings of recoveries for evaluation.

Robustness [26-32]

The method was authenticated by the examination of variance (ANOVA). The numerical examination illustrated (Table 6) that the method symbolizes the occurrence excellently and the difference of the reaction was accurately connected to the difference of the features. The ANOVA chart obtained is a synopsis of the importance of the worldwide method. If p-value for the worldwide method is lesser than 0.05, it discloses the method is noteworthy at 5 % level. That is a lower P-value the additionally important is the method. As the p vales obtained are more than 0.05 null hypothesis Ho is accepted. The effect summary is reported in Table 8 which offers an outline of the importance of every impact for all reactions.

The regression equation model for MFN, ATR and is in equation 2, 3 and 4

$$Y_{MFN} = 99.50 + 0.04X_1 - 0.22\,X_2 + 0.08X_3 + 0.10\,X_1X_2 + 0.27\,X_1X_3 + 0.09\,X_2X_3 + 0.26\,X_1X_2X_3$$

Equation -2

$$Y_{ATR} = 99.28 + 0.01X_1 - 0.04\,X_2 - 0.10X_3 + 0.49X_1X_2 + 0.10\,X_1X_3 + 0.03\,X_2X_3 - 0.02\,X_1X_2X_3$$

Equation -3

$$Y_{GPE} = 99.45 + 0.11X_1 - 0.04\,X_2 + 0.23X_3 + 0.26\,X_1X_2 - 0.11\,X_1X_3 + 0.05X_2X_3 - 0.26\,X_1X_2X_3$$

Equation - 4

In conclusion, by examining the ANOVA results confirms that Y_{MFN}, Y_{ATR} and Y_{GPE} are robust for all the three factors.

Table 2: System suitability report

Analyte	Retention Time (R_t)	USP resolution (R_s)	USP Tailing (T)	No. of Theoretical plates USP Tangent Method (N)
MFN	0.393	----	0.82	10727
ATR	1.380	14.5	0.93	9039
GPE	1.625	3.05	1.02	8142

Table 3: Intraday and interday Precision results of MFN, ATR and GPE from tablets

S.no	MFN		ATR		GPE	
	Intra Assay	Inter Assay	Intra Assay	Inter Assay	Intra Assay	Inter Assay
1	99.21	98.62	99.59	101.22	99.56	100.25
2	99.52	99.59	99.54	100.21	99.43	100.06
3	99.62	99.48	99.85	100.35	99.68	99.56
4	99.79	100.26	100.12	99.65	99.95	98.52
5	98.65	101.48	99.65	100.56	99.56	99.21
6	100.52	99.24	99.67	99.56	99.73	99.82
Mean	99.55	99.78	99.74	100.26	99.65	99.57
%RSD	0.62	0.99	0.22	0.61	0.18	0.63
Grand Mean	99.67		100.00		99.61	

%RSD	0.80	0.52	0.45
S.E	0.23	0.15	0.13

Table 4: Results of Linearity study

Parameters	MFN	ATR	GPE
Calibration equation	y = 4147x + 661	y = 63278 X - 498	Y = 32838X + 191
Linearity range	70 – 130 %	70 – 130 %	70 – 130 %
Regression coefficient	0.9992	0.9992	0.9995
slope	4147	63278	32838
Intercept	661	-498	191

Table 5: Results of Accuracy

Recovery level	MFN			ATR			GPE		
	Amount taken (mg)	Amount found (mg)	% Recovery	Amount taken (mg)	Amount found (mg)	% Recovery	Amount taken (mg)	Amount found (mg)	% Recovery
80%	400	399.04	99.76	8	7.96	99.54	1.6	1.61	100.63
(n=3)	400	397.24	99.31	8	7.95	99.43	1.6	1.59	99.60
	400	398.76	99.69	8	7.99	99.85	1.6	1.58	99.31
100% (n=3)	500	500.35	100.11	10	10.02	100.22	2	1.99	99.55
	500	495.95	99.19	10	9.99	99.95	2	1.98	99.02
	500	496.80	99.36	10	10.03	100.32	2	1.99	99.92
120% (n=3)	600	601.56	100.26	12	12.04	100.41	2.4	2.41	100.39
	600	596.04	99.34	12	11.96	99.72	2.4	2.39	99.62
	600	598.38	99.73	12	11.91	99.29	2.4	2.37	98.73
Mean			99.64			99.86			99.58
SD			0.37			0.40			0.60
% RSD			0.37			0.40			0.61
95% Confidence Interval			+ 0.24			+0.13			+ 0.40

Table 6: ANOVA results

Parameter	SS	DF	MS	F	P
Flow rate (µL min^{-1})	0.0968	1	0.0968	0.1868	0.69
Acetonitrile (%)	0.0128	1	0.0128	0.0247	0.88
pH	0.4232	1	0.4232	0.8167	0.43

Table 7: Experimental plans for robustness testing and obtained responses

Experiment no.	Flow rate (µL min^{-1})	Acenonitrile (%)	pH	Assay (%)		
				MFN	ATR	GPE
1	1	1	-1	98.56	99.52	99.83
2	1	-1	-1	97.52	98.63	97.96
3	0	0	0	99.92	99.85	99.89
4	1	1	1	99.99	99.52	99.65
5	-1	1	1	98.62	98.35	99.65
6	1	-1	1	99.53	98.62	99.62
7	0	0	0	99.84	99.62	98.62
8	-1	-1	1	98.62	99.32	98.62
9	-1	-1	-1	99.65	99.87	99.58
10	-1	1	-1	99.33	98.66	98.33
11	0	0	0	100.02	100.12	100.26

* Randomized

Table 8: Effect summaries

Effects	MFN			ATR			GPE		
	Significance	Effect value	P -value	Significance	Effect value	P - value	Significance	Effect value	P - value
X1 (A)	NS	0.09	0.80	NS	0.02	0.96	NS	0.22	0.69
X2 (B)	NS	-0.45	0.28	NS	-0.09	0.85	NS	-0.08	0.88
X3 (C)	NS	0.17	0.65	NS	-0.21	0.69	NS	0.46	0.43
X1 * X2 (AB)	NS	0.20	0.59	NS	0.99	0.14	NS	0.53	0.37
X1 * X3 (AC)	NS	0.54	0.21	NS	0.21	0.70	NS	-0.22	0.69
X2 * X3 (BC)	NS	0.18	0.63	NS	0.06	0.90	NS	0.11	0.84
X1 * X2 * X3 (ABC)	NS	0.52	0.23	NS	-0.05	0.91	NS	-0.53	0.37

CONCLUSION

The selected analytes such as MFN, ATR and GPE has been simultaneously analyzed in pharmaceutical formulation (tablet) with UHPLC. The entire run time happened to be 4 min, wherein the three peaks MFN, ATR and GPE were well separated. The proposed rapid UHPLC method had been assessed on the linearity, accuracy, precision, specificity and robustness and established to be suitable and effectual in the quality assessment of MFN, ATR as well as GPE in Pharmaceutical tablet hence can be used in quality control laboratories for the estimation of MFN, ATR and GPE. The findings of the research reveal the advantage of utilizing experimental design based robustness study in method validation.

ACKNOWLEDGEMENT

The authors are highly grateful to the management of SRM College of Pharmacy, SRM University, Chennai and Ideal Analytical and Research Institution, Villianur, Puducherry, for offering conveniences to conduct the study.

REFERENCES

1. American Diabetes Association. Standards of medical care for patients with diabetes mellitus (Position Statement). Diabetes Care 2003; 26: 33–50.
2. A.J. Krentz and C.J. Bailey. Oral antidiabetic agents: current role in type 2 diabetes mellitus. Drugs 2005; 65: 385–411.
3. R.A. DeFronzo, N. Barzilai and D.C. Simonson. Mechanism of MFN action in obese and lean noninsulin-dependent diabetic subjects. J. Clin. Endocrinol. Metab. 1991; 73: 1294– 1301.
4. C.J. Bailey and R.C. Turner. Metformin. Engl. J. Med. 1996; 334: 574–579.
5. R. Rosskamp, K. Wernicke–Panten and E. Draeger. Clinical profile of the novel sulphonylurea glimepiride. Diabetes Res. Clin. Pract. 1996; 31:33–42.
6. M. Massi-Benedetti. Glimepiride in type 2 diabetes mellitus: a review of the worldwide therapeutic experience. Clin. Ther. 2003; 25: 799–816.
7. Bakker-Arkema RG, Davidson MH, Goldstein RJ, Davignon J, Isaacsohn JL, Weiss SR, Keilson LM, Brown WV, Miller VT, Shurzinske LJ and Black DM. Efficacy and safety of a new HMG-CoA reductase inhibitor, atorvastatin, in patients with hypertriglyceridemia. J. Am. Med. Assoc. 1996; 275: 128–133.
8. Nawrocki JW, Weiss SR, Davidson MH, Sprecher DL, Schwartz SL, Lupien PJ, Jones PH, Haber HE and Black DM, Reduction of LDL cholesterol by 25% to 60% in patients with primary hypercholesterolemia by atorvastatin, a new HMG-CoA reductase inhibitor. Arteriosclerosis, Thromb. Vasc. Biol. 1995; 15:678-682.
9. Taskinen MR. Strategies for the management of diabetic dyslipidemia. Drugs 1998; 58: 47–51.
10. Zimmet P, Collier G. Clinical efficacy of MFN against insulin resistance parameters. Drugs 1998; 58: 21–28.
11. F. S. Bandarkar and I. S. Khattab. Simultaneous estimation of glibenclamide, gliclazide, and metformin hydrochloride from bulk and commercial products using a validated ultra fast liquid chromatography technique. J. Liq. Chromatogr. Related Technol. 2010; 33: 1814-1830. doi: 10.1080/10826076.2010.532704.
12. Chellu S.N.Malleswararao, Mulukutla V.Suryanarayana and Khagga Mukkanti. Simultaneous determination of Sitagliptin Phospahte Monohydrate and Metformin Hydrochloride in tablets by a validated UPLC method. Sci. Pharm. 2012; 80: 139-152. doi: 3797/scipharm.1110-13.
13. Vora, D. N and Kadav, A. A Validated Ultra HPLC method for the simultaneous determination of Atorvastatin, Aspirin and their degradation products in capsules. J. Liq. Chromatgr. Related Technol. 2008; 31:2821-2837.
14. Raja Kumar Seshadri, Makarand Madhukar desai, Thummala Veera raghavaraju, Deepa Krishnan, Dama venugopala rao and Ivon elisha chakravarthy. Simultaneous Quantitative Determination of Metoprolol, Atorvastatin and Ramipril in Capsules by a Validated Stability-Indicating RP-UPLC Method, Sci. Pharm. 2010; 78: 821–834.
15. H. O. Kaila, M. A. Ambasan and A.K Shah. A Simple and Rapid Ultra Performance Liquid Chromatographic Assay Method for the Simultaneous determination of aspirin, clopidogrel bisulphate and atorvastatin Calcium in capsule dosage form. International Journal of Chem Tech Research. 2011; 3: 459-465.
16. Satheesh Kumar Shetty, K. V. Surendranath. Stress Degradation Behavior of a Polypill and Development of Stability Indicating UHPLC Method for the Simultaneous Estimation of Aspirin, Atorvastatin, Ramipril and Metoprolol Succinate. Ame. J. of Ana. Chem. 2011; 2: 401-410.
17. Ramesha B, K.R. Venugopala reddy, Unni Krishnan.M, Vidyanand ankolekar, Anuradha.P and Amith kumarM.K. A validated UHPLC method for the determination of atorvastatin acetonide tert – butyl ester and 4-fluoro-alpha - (2-methyl-1-oxopropyl) – gamma – oxo-n, beta – diphenyl benzene butane amide, Asian J. of Pharm. And Clin. Res. 2012; 5: 115-122.
18. Seshukumar Devu, Abhishek Gupta, Kona S Srinivas, Ravi Shankar Gupts and Vinod Prasad Semwal. Development and Validation of Stability indicating RP-UPLC method for simultaneous determination in fixed dose combination of ezetimibe and simvastatin. J. Chromat. Seperation Techni. 2012; 3: 131.
19. Kakumani Kishore Kumar, Chimalakonda Kameswara Rao, Maddala Vijaya Lakshmi and Khagga Mukkanti. A Validated stability indicating RP-UPLC method for atorvastatin Calcium. Ame. J. of Ana. Chem. 2012; 3: 392-399.
20. Thummala Veera Raghavaraju, Deepa Krishnan, B. V. Kishore,,M. K. Amith Kumar, L. P. Raju abd B. N. Thara. Development and validation of a stability indicating gradient RP-UHPLC method for the determination of impurities in atorvastatin drug substance. J. Liq. Chromatogr. Related Technol. 2013.
21. Mallikarjuna S, Ramalingam P, Sriram P, Garima J and Srinivas SK. Development and Validation of stability indicating RP-UPLC method for simultaneous estimation of amlodipine besylate, and atorvastatin calcium in pharmaceutical dosage forms. J. Liq. Chromatogr. Related Technol. 2013; 4: 187.
22. Goel A, Baboota S, Sahni JK, Srinivas KS, Gupta RS, Gupta A, Semwal VP and Ali J. Development and validation of stability indicating assay method by UPLC for a fixed dose combination of atorvastatin and ezetimibe, J Chromatogr Sci. 2013; 51**:** 222-228. doi: 10.1093/chromsci/bms131.
23. Lakshmi Narasimham, Y. S and Barhate, Vasant D Barhate. Development and validation of stability indicating UPLC method for the simultaneous determination of antidiabetic drugs in pharmaceutical dosage forms. Journal of Pharmacy Research 2010; 3: 3081-3087.
24. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonized Tripartite Guidance: Validation of Analytical Procedures:Text and Methodology Q2 (R1), ICH. London 2005.
25. Vander H Y, Nijhuis A, Smeyers V J, Vandeginste BG, Massart DL. Guidance on robustness/ ruggedness tests in method validation. J. Pharm. Biomed. Anal. 2001; 24:723-753.
26. D. K. Lin. Discussion on papers by Box and Liu, Box and Myers. J. of Qua. Tech. 1999; 31: 61–66.
27. K. K. Hockman and D. Berengut. Design of experiments. Chemical Engineering. 1995; 102: 142–148.
28. H. Fabre. Robustness testing in Liquid Chromatography. J. Pharm. Biomed. Anal. 1996; 14: 1125–1132.
29. G.A. Lewis, D. Mathieu and R. Phan-Tan-Luu. Pharmaceutical Experimental Design Marcel Dekker, New York, NY, USA 1999.
30. S. Pinzauti, P. Gratteri, S. Furlanetto, P. Mura, E. Dreassi, R. Phan-Tan-Luu. Experimental design in the voltammetric method for the assay of omeprazole. J. Pharm. Biomed. Anal. 1996; 14: 881–889.
31. Sanjay s. patel, Natvarlal m. patel. Development of directly compressible co-processed excipient for dispersible tablets using 32 full factorial design. International journal of pharmacy and pharmaceutical sciences. 2009; 1:125-148.
32. Ravichandran v, Shalini s, Sundram k. m and Sarish raja, Validation ofanalytical methods – strategies &importance. International journal of pharmacy and pharmaceutical sciences. 2010; 2: 18-22.