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Research Article

Develop a new, simple, fast, rapid, accurate, efficient and Reproducible RP-UPLC Method for the Simultaneous analysis of Metformin and Saxagliptin

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Abstract

Introduction: Metformin decreases blood glucose levels by decreasing hepatic glucose production, decreasing intestinal absorption of glucose, and improving insulin sensitivity by increasing peripheral glucose uptake and utilization. These effects are mediated by the initial activation by metformin of AMP-activated protein kinase (AMPK), a liver enzyme that plays an important role in insulin signaling, Saxagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor antidiabetic for the treatment of type 2 diabetes. **Aim and Objectives:** The present work is aimed to develop a new, simple, fast, rapid, accurate, efficient and reproducible RP-UPLC method for the simultaneous analysis of Metformin and Saxagliptin. The developed method will be validated according to ICH guidelines. **Methods:** In our study we used chemicals were water, Methanol, Acetonitrile, Ortho phosphoric acid, KH_2PO_4, K_2HPO_4, 0. 22µ Nylon filter, 0.45µ filter paper, RCm XR, Metformin and Saxagliptin. The Instruments UPLC-auto sampler–PDA detector, U.V double beam spectrometer, Digital weighing balance (sensitivity 5mg), pH meter, Sonicator were used. **Results and Discussion:** The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Metformin and Saxagliptin was found in concentration range of 50µg-250µg and 5µg-25µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.8 and 0.3, % RSD for intermediate precision was 1.1 and 0.3 respectively. The precision study was precision, robustness and repeatabilty. LOD value was 2.17 and 0.0372 and LOQ value was6.60and 0.1125 respectively. **Conclusion:** The study suggested RP-UPLC method can be used for routine analysis of Metformin and Saxagliptinin API and Pharmaceutical dosage form.

Keywords: Metformin, Saxagliptin, RP-UPLC, ICH guidelines, LOD, LOQ, Pharmaceutical dosage form.

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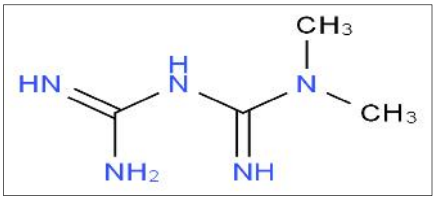
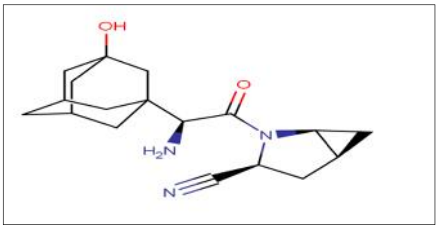
1. Introduction



Metformin



Saxagliptin

Metformin decreases blood glucose levels by decreasing hepatic glucose production, decreasing intestinal absorption of glucose, and improving insulin sensitivity by increasing peripheral glucose uptake and utilization. These effects are mediated by the initial activation by metformin of AMP-activated protein kinase (AMPK), a liver enzyme that plays an important role in insulin signaling, Saxagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor antidiabetic for the treatment of type 2 diabetes. DPP-4 inhibitors are a class of compounds that work by affecting the action of natural hormones in the body called incretins. Incretins decrease blood sugar by increasing consumption of sugar by the body, mainly through increasing insulin production in the pancreas, and by reducing production of sugar by the liver[1-3].

2. Materials and Methods

Table 1: List of chemicals and standards used

S.No	Chemicals	Manufacturer Name	Grade
1.	Water	Merck	HPLC grade
2.	Methanol	Merck	HPLC grade
3.	Acetonitrile	Merck	HPLC grade
4.	Ortho phosphoric acid	Merck	G.R
5.	KH_2PO_4	Merck	G.R
6.	K_2HPO_4	Merck	G.R
7.	0. 22µ Nylon filter	Advanced lab	HPLC grade
8.	0.45µ filter paper	Millipore	HPLC grade
9.	RCm XR	Mankind	Tablet form
10.	Metformin and Saxagliptin	In – House	In- House

Table 2: List of instruments used

S.No	Instrument name	Model number	Soft ware	Manufacturers Name
1	UPLC-auto sampler –PDA detector	Acquity Model-996 PDA	Empower-software version-2	Waters
2	U.V double beam spectrometer	UV 3000+	U.V win soft ware	Lab India
3	Digital weighing balance(sensitivity 5mg)	ER 200A	-	Ascoset
4	pH meter	AD 102U	-	ADWA
5	Sonicator	SE60US	-	Enertech

Preparation of phosphate buffer:

2.95 grams of $KH_2PO_4$and 5.45 grams of K_2HPO_4 was weighed and taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water and pH was adjusted to 3 with orthophosphoric acid. The resulting solution was sonicated and filtered.

Preparation of mobile phase

Mix a mixture of above buffer 300 ml (30%) and 700 ml of methanol (HPLC grade-70%) and degassed in ultrasonic water bath for 5 minutes. Filter through 0.22 µ filter under vacuum filtration.

Diluents preparation

Mobile phase was used as the diluent.

Preparation of the individual Metformin standard preparation: 10 mg of Metformin working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1.5ml from the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Preparation of the individual Saxagliptin standard preparation: 1 mg of Saxagliptin working standard was accurately weighed and transferred into a 10ml clean dry

volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette out 3 ml from the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Preparation of the Metformin and Saxagliptin standard and sample solution

Sample solution preparation: 10mg of Metformin and 1mg Saxagliptin tablet powder were accurately weighed and transferred into a 10ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

Standard solution preparation

10 mg Metformin and 1mg Saxagliptin working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent[8-9].

3. Results and Discussion

The retention time of Metformin and Saxagliptin was found to be 0.506mins and 0.702mins respectively. The system suitability parameters for Metformin and Saxagliptin such as theoretical plates and tailing factor were found to be 2294, 1.27and 4891, 1.03. Resolution was8.67.The % purity Metformin and Saxagliptinin pharmaceutical dosage form was found to be 98.24 and 100.27% respectively.

Validation Report

Specificity

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak.

Accuracy

The accuracy study was performed for 50%, 100% and 150 % for metformin and Saxagliptin. Each level was injected in triplicate into chromatographic system.

Robustness

The robustness was performed for the flow rate variations from 0.27ml/min to 0.33ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for metformin and Saxagliptin. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The results are summarized on evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±0.03ml/min. The method is robust only in less flow condition[10-11].

Discussion: The present investigation reported in the thesis was aimed to develop a new method development and validation for the simultaneous estimation of Metformin and Saxagliptin by RP-UPLC method. Literature reveals that there are no analytical methods reported for the simultaneous estimation Metformin and Saxagliptin by RP-UPLC method. Hence, it was felt that, there is a need of new analytical method development for the simultaneous estimation of Metformin and Saxagliptin in pharmaceutical dosage form.

Table 3: Assay calculation for metformin and Saxagliptin

S.No	Linearity Level	Concentration	Area
1	I	50 ppm	800199
2	II	100 ppm	1589391
3	III	150 ppm	2264300
4	IV	200 ppm	3071625
5	V	250 ppm	3894075
Correlation Coefficient			0.999

Table 4: Showing accuracy results for metformin

%Concentration (at specification level)	Average area	Amount added(mg)	Amount found (mg)	% Recovery	Mean recovery
50%	1184204.3	5	4.96	99.91%	99.56%
100%	2121872.4	10	9.98	99.18%	
150%	3525766.1	15	15.02	99.60%	

Table 5: Showing accuracy results for Saxagliptin

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	52228.2	0.5	0.99	99.53%	99.47%
100%	979319	1.0	1.05	99.38%	
150%	1576651	1.5	1.495	99.52%	

Table 6: Showing precision results

Injection	Area for Metformin	Area for Saxagliptin
Injection-1	2270553	993413
Injection-2	2278100	993859
Injection-3	2282356	998213
Injection-4	2283157	998930
Injection-5	2285975	999663
Injection-6	2284862	998652
Average	**2280833.8**	**997121.7**
Standard Deviation	**5719.1**	**2744.5**
%RSD	**0.3**	**0.3**

Table 7: Showing results for Limit of Detection

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Metformin	371827.90	563365963	2.17
Saxagliptin	5401.60	479884400	0.0372

Table 8: Showing results for Limit of Quantitation

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Metformin	371827.90	563365963	6.60
Saxagliptin	5401.60	479884400	0.112

Molecular weight : Average: 206.2808 Monoisotopic: 206.13067982 g/mol.

Bioavailability : 87–100% (oral), 87% (rectal)

Half-life : 2-4 hours

Protein binding : 90-99% to whole human plasma and site II of purified albumin, binding appears to be saturable and becomes non-linear at concentrations exceeding 20mcg/ml.

Dosage forms: tablet, solution, injection.

Dose : 200,400,600,800mg. 10,100mg/ml/5ml

Category : Anti-Inflammatory Agents, Non-Steroidal, Cyclooxygenase Inhibitors, Analgesics, Non-Narcotic.

Pharmacodynamics:

Ibuprofen is a nonsteroidal anti-inflammatory agent (NSAIA) or nonsteroidal anti-inflammatory drug (NSAID), with analgesic and antipyretic properties. Ibuprofen has pharmacologic actions similar to those of other prototypical NSAIAs, which are thought to act through inhibition of prostaglandin synthesis[1].

Mechanism of Action:

The exact mechanism of action of ibuprofen is unknown. Ibuprofen is a non-selective inhibitor of cyclooxygenase, an enzyme invovled in prostaglandin synthesis via the arachidonic acid pathway. Its pharmacological effects are believed to be due to inhibition cylooxygenase-2 (COX-2) which decreases the synthesis of prostaglandins involved in mediating inflammation, pain, fever and swelling. Antipyretic effects may be due to action on the hypothalamus, resulting in an increased peripheral blood flow, vasodilation, and subsequent heat dissipation. Inhibition of COX-1 is thought to cause some of the side effects of ibuprofen including GI ulceration.

Pharmacokinetic Properties:

Absorption: ~ 80% absorbed from GI tract, Time to reach peak plasma concentration = 47 minutes (suspension), 62 minutes (chewable tablets), 120 minutes (conventional tablets)

Distribution:

Ibuprofen, like most drugs of its class, is highly protein bound (>99% bound at 20 µ g/mL). Protein binding is saturable and at concentrations >20 µ g/mL binding is non-linear. Based on oral dosing data there is an age- or fever-related change in volume of distribution for ibuprofen. Febrile children <11 years old have a volume of approximately 0.2 L/kg while adults have a volume of approximately 0.12 L/kg. The clinical significance of these findings is unknown.

Metabolism:

R-enanatiomer undergoes extensive enantiomeric conversion (53-65%) to the more active S-enantiomer in vivo. Metablized by oxidation to 2 inactive metabolites: (+)-2[4´-(2-hydroxy-2-methylpropyl) phenyl] propionic acid and (+)-2-[4´-(2-carboxypropyl) phenyl] propionic acid. Very small amounts of 1-hydroxyibuprofen and 3-hydroxyibuprofen have been recovered from urine. Cytochrome P450 2C9 is the major catalyst in the formation of oxidative metabolites. Oxidative metabolites may be conjugated to glucuronide prior to excretion.

Elimination:

Ibuprofen is rapidly metabolized and eliminated in the urine. The excretion of ibuprofen is virtually complete 24 hours after the last dose. It has a biphasic plasma elimination time curve with a half-life of approximately 2.0 hours. There is no difference in the observed terminal elimination rate or half-life between children and adults, however, there is an age-or fever-related change in total

clearance. This suggests that the observed change in clearance is due to changes in the volume of distribution of ibuprofen[2-5].

Adverse Effects:

Nausea, dyspepsia, gastrointestinal ulceration/bleeding, raised liver enzymes, diarrhea, constipation, nosebleed, headache, dizziness, rash, salt and fluid retention, and hypertension. Infrequent adverse effects include: esophageal ulceration, heart failure, hyperkalemia, renal impairment, confusion, and bronchospasm. Ibuprofen can exacerbate asthma, sometimes fatally.

Storage: Store at room temperature

Oxaprozin

IUPAC Name: potassium 3-(4,5-diphenyl-1,3-oxazol-2-yl) propanoate

Chemical formula: $C_{18}H_{14}KNO_3$

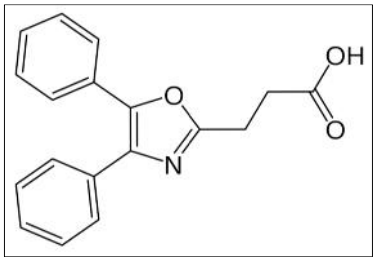
Molecular weight : 331.412

Cas No : 174064-08-5

Category : Analgesics, Non-Narcotic, Anti-Inflammatory Agents.

Mechanism of action:

Anti-inflammatory effects of Oxaprozin are believed to be due to inhibition of cylooxygenase in platelets which leads to the blockage of prostaglandin synthesis. Antipyretic effects may be due to action on the hypothalamus, resulting in an increased peripheral blood flow, vasodilation, and subsequent heat dissipation. Oxaprozin is a non-selective NSAID, with a cell assay system showing lower COX-2 selectivity implying higher COX-1 selectivity.

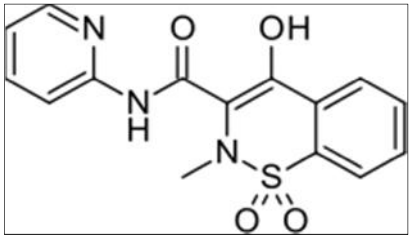


Brand name: Daypro

Piroxicam

IUPAC Name: 2-methyl-1, 1-dioxo-3-[(pyridin-2-yl) carbamoyl]-2H-1lambda6,2-benzothiazin-4-yl (2E)-3-phenylprop-2-enoate

Chemical formula: $C_{24}H_{19}N_3O_5S$



Molecular weight: 461.49

Cas No : 90101-16-9

Category: Agents causing hyperkalemia, Agents that produce hypertension.

Mechanism of action: Droxicam is converted to Piroxicam via hydrolysis of the ester group in the intestine 2. Droxicam administration inhibits the synthesis of prostaglandins by cyclooxygenase enzymes.

Brand name: Feldene

2. Materials and Methods

Selection of wavelength

10 mg of Ibuprofen, Oxaprozin and Piroxicam was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained. The overlay spectrum was used for selection of wavelength for Ibuprofen, Oxaprozin and Piroxicam. The isobestic point was taken as detection wavelength.

Selection of column

Heart of HPLC made of 316 grade stainless steel packed with stationary phase. Silica based columns with different cross linking's in the increasing order of polarity are as follows:

Non-polar----------moderately polar--------Polar

C_{18}< C_8< C_6< Phenyl < Amino <Cyano< Silica

- In reverse phase chromatography, hydrophobic interaction between drug molecule and the alkyl chains on the column packing material.
- Column is selected based on solubility, polarity and chemical differences among analysts and Column selected: i.e Agilent (4.6×150mm)5µ.
- Reasons : Better separation,
  Good tailing factor.

Selection of solvent delivery system

- Always preferable solvent delivery system.
- More chance of getting reproducible result on retention time of analytes.
- More economic than gradient technique.

Selection of flow rate

Acceptable limit: - Not more than 2.5 ml/min

- Flow rate selected was 1ml/min
- Flow rate is selected based on
  1. Retention time
  2. Column back pressure
  3. Peak symmetry.
  4. Separation of impurities.

Reasons:

- For earlier elution of analyte and elution of all impurities within 6.0 min.
- Information from the reference method in literature.

Selection of diluent

- Selection of diluent is based on the solubility of the analyte
- Diluent selected: Methanol : phosphate buffer pH 3 (55 : 45v/v)

Reason: good peak area, retention time, peak symmetry

Selection of column temperature:

- Preferable temperature is ambient or room temperature.

Reasons:

- To elute all impurities along with analyte with in 10.0 min of run time.
- Less retention time
- Good peak shape
- Higher theoretical plates.
- Good resolution.

Selection of test concentration and injection volume

Test concentration is finalized after it is proved that API is completely extractable at the selected test concentration.

- Test concentration is fixed based upon the response of API peak at selected detector wavelength.
- And the test concentration selected is 10 ppm.
- Injection volume selected was 10µL.

3. Results and Discussion

Table 1: Chromatogram values for System suitability of Ibuprofen

Injection	R_t	Peak Area	USP Plate count	USP Tailing
1	2.405	1250763	2487	1.62
2	2.406	1247867	2489	1.58
3	2.406	1255849	2496	1.64
Mean		1251360		
SD		3850.679		
% RSD		0.30722		

Acceptance Criteria:

1). Tailing factor Obtained from the standard injection is 1.7

2). Theoretical Plates Obtained from the standard injection is 2496

Table no 2: Chromatogram values for System suitability of Oxaprozin

Injection	R_t	Peak Area	USP Plate count	USP Tailing	USP Resolution
1	2.417	940627	2271	1.52	3.02
2	2.418	931161	2243	1.46	3.07
3	2.417	940306	2262	1.45	3.04
Mean		937364.7			
17SD		5374.93			
% RSD		0.473409			

Acceptance Criteria:

1) Tailing factor Obtained from the standard injection is 1.51

2) Theoretical Plates Obtained from the standard injection is 2281

Table no-3: Chromatogram values for System suitability: **Piroxicam**

Injection	R_t	Peak Area	USP Plate count	USP Tailing	USP Resolution
1	7.087	933579	2504	1.22	11.84
2	7.088	934565	2592	1.23	12.23
3	7.087	920436	2564	1.24	13.14
Mean		929157.7			
SD		7597.932			
% RSD		0.827823			

Acceptance Criteria:

1) Tailing factor Obtained from the standard injection is 1.25

2) Theoretical Plates Obtained from the standard injection is 2594

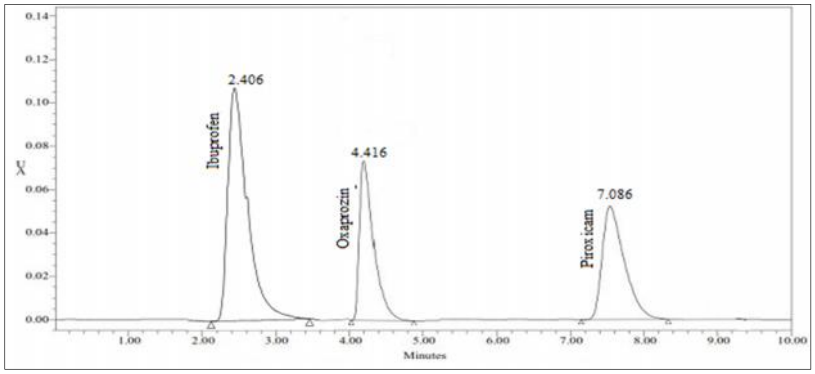


Fig.No.1. Chromatogram for Linearity level 2

Table 4: Linearity results for Ibuprofen

S.No	Linearity Level	Concentration(µg/ml)	Area
1	I	10 ppm	339286
2	II	20 ppm	667774
3	III	30 ppm	986474
4	IV	40 ppm	1339994
5	V	50 ppm	1639065
Correlation Coefficient			0.999

Table 5: Linearity results for Piroxicam

S.No	Linearity Level	Concentration (µg/ml)	Area
1	I	12.5	231737
2	II	25	453615
3	III	37.5	659796
4	IV	50	895191
5	V	62.5	1094356
Correlation Coefficient			0.999

Table no-6: Calibration parameters for Ibuprofen, Oxaprozin and Piroxicam

Parameter	Results for Ibuprofen	Results for Oxaprozin	Results for Piroxicam
Slope	18718	14315	70355
Intercept	65497	49120	47086
Correlation co-efficient	0 .9993	0.99918	0.99902

Acceptance criteria:

1. Correlation Coefficient should be not less than 0.9990.
2. % RSD of peak areas for Solution 1, 2, 3, 4 and 5 should be not more than 2.0 %.

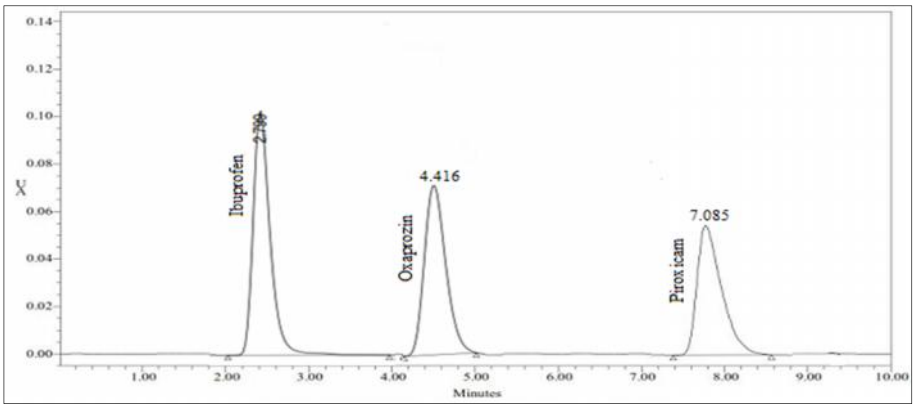


Fig.No.2. Sample Chromatograms for precision injection-1

Table 7: Sample Chromatogram values for Repeatability Ibuprofen

Injection No	Peak Area	R_t
1	935035	4.416
2	929353	4.417
3	930459	4.619
4	932389	4.418
5	922057	4.417
Avg	927458.6	
SD	4865.16	
% RSD	0.4232	

Table no-8: Chromatogram values for intermediate Precision: Ibuprofen

Injection No	Peak Area	R_t
1	912412	4.416
2	913062	4.417
3	909642	4.418
4	916881	4.419
5	914005	4.418
Mean	913200.4	
SD	2621.886	
% RSD	0.387	

Table no-9: Chromatogram values for intermediate Precision: Oxaprozin.

Injection No	Peak Area	R_t
1	914922	7.086
2	909335	7.085
3	913266	7.086
4	909418	7.087
5	911496	7.086
Mean	911687.4	
SD	2432.859	
% RSD	0.4668	

Table no-10: Chromatogram values for intermediate Precision: Piroxicam .

Sample No.	Spike Level	Amount added(mg)	Amount found(mg)	% Recovery	Mean % Recovery
1	50 %	5	4.9	98%	100%
		5	5.1	102%	
		5	5	100%	
2	100 %	10	9.88	98.8%	99.31%
		10	9.91	99.1%	
		10	9.95	99.5%	
3	150 %	15	14.89	99.2%	99.89%
		15	14.86	99.0%	
		15	14.99	99.79%	

Table 11: Chromatogram Values for Accuracy of Ibuprofen.

Sample No.	Accuracy	Amount added(mg)	Amount found(mg)	% Recovery	Mean % Recovery
1	50 %	5	4.9	98%	100%
		5	5.1	102%	
		5	5	100%	
2	100 %	10	9.88	98.8%	99.13%
		10	9.91	99.1%	

		10	9.95	99.5%	
3	150 %	15	14.89	99.2%	99.69%
		15	14.86	99.0%	
		15	14.82	99.79%	

Table 12: Chromatogram Values for Accuracy of Oxaprozin

Sample No.	Spike Level	Amount added(mg)	Amount found(mg)	% Recovery	Mean % Recovery
1	50 %	10	9.8	98%	100%
		10	10.2	102%	
		10	10	100%	
2	100 %	20	19.8	99%	100%
		20	20.2	101%	
		20	20	100%	
3	150 %	30	29.6	98.66%	99.33%
		30	30	100%	
		30	29.8	99.33%	

Table 13: Robustness results for Ibuprofen (flow rate)

S.No	Drug	Flow Rate ml/min		
		0.8ml/min R_t	1ml/min R_t	.1.2m l/min R_t
1	**Oxaprozin Robustness Results**	2.475	2.482	2.488
JSP Plate count		2279	2232	2185
USP Tailing		1.47	1.49	1.51

Table 14: Robustness results for Oxaprozin (flow rate):

S.No	Drug	Flow Rate ml/min		
		0.8ml/min R_t	1ml/min R_t	1.2 m l/min R_t
1	**Piroxicam Robustness Results**	3.488	3.190	2.877
USP Plate count		2346	2556	2096
USP Tailing		1.28	1.24	1.27

Table 15: Robustness results for Oxaprozin

S.No	Drug	Mobile phase		
		More organic (R_t)	Organic (R_t)	ess organic (R_t)
1	**Piroxicam Robustness Results**	7.087	7.086	7.086
USP Plate count		2482	2556	2030
USP Tailing		1.21	1.23	1.32

Table no-14: Results of LOD

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Ibuprofen	371877.10	563365963	2.03
Oxaprozin	431401.80	476884400	0.0365
Piroxicam	287058.10	376884400	1.84

Table no-15: Results of LOQ

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Ibuprofen	371877.10	563365963	4.53
Oxaprozin	431401.80	476884400	5.75
Piroxicam	287058.10	376884400	3.84

LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula.

4. Conclusion

A new method was established for simultaneous estimation of Metformin and Saxagliptinby RP-UPLC method. The chromatographic conditions were successfully developed for the separation of Metformin and Saxagliptin by using Dikma Endeversil C18 column (4.6×50mm) 3µm, flow rate was 0.3 ml/min, mobile phase ratio was (70:30 v/v) methanol: phosphate buffer ($KH_2PO_4$and K_2HPO_4) phosphate pH 3(pH was adjusted with ortho phosphoric acid),detection wavelength was 236 nm. The instrument used was WATERS Acquity Model UPLC^{TM}e, photo diode array detector 996, Empower-software version-2. The retention times were found to be 0.502mins and 0.706mins. The % purity of Metformin and Saxagliptin was found to be 99.87% and 100.27% respectively[12-13]. The system suitability parameters for Metformin and Saxagliptin such as theoretical plates and tailing factor were found to be 2294, 1.27 and 4891 and 1.03, the resolution was found to be 8.67. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Metformin and Saxagliptin was found in concentration range of 50µg-250µg and 5µg-25µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.8 and 0.3, % RSD for intermediate precision was 1.1 and 0.3 respectively. The precision study was precision, robustness and repeatabilty.LOD value was 2.17 and 0.0372 and LOQ value was6.60and 0.1125 respectively. Hence the suggested RP-UPLC method can be used for routine analysis of Metformin and Saxagliptinin API and Pharmaceutical dosage form.

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