AN OVERVIEW ON METHODS VALIDATION OF SIMULTANEOUS
ESTIMATION FOR METFORMIN HCL AND SITAGLIPTIN
PHOSPHATE MONOHYDRATE IN TABLET DOSAGE FORM

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ABSTRACT
A rapid, sensitive and specific methods of validation for estimation of
metformin hcl and sitagliptin phosphate monohydrate in bulk and tablet
dosage formulation. The methods were validated in terms of linearity,
accuracy, assay, precision, specificity, limit of detection and limit of
quantitation. The optimum conditions for the analysis of the drug were
established.

KEYWORDS: Sitagliptin, Metformin, Simultaneous estimation,
Validation.

INTRODUCTION
Diabetes mellitus is often a diabetes that is a group of common
endocrine diseases distributed by sustained increase in blood sugar
levels. It is due to the pancreas that doesn't produce enough insulin.
Basic symptoms include thirst, polyuria, weight loss and blurred
vision. If left untreated then diabetes may lead to various health issues like disorders of the
cardiovascular system, eye, kidney and nerves, etc.[1]

Types of diabetes
Type - I diabetes
Gestational diabetes
Type - II diabetes
Prediabetes

1. Type 1 diabetes: In which the pancreas does not produce the insulin that is essential for survival. This form develops most commonly in children and adolescents. (insulin dependent diabetes mellitus).

2. Type 2 diabetes: which results from the body's inability to properly respond to the effects of insulin produced by the pancreas. Type 2 diabetes is much more common, accounting for about 90% of all diabetes cases worldwide. (formerly, non-insulin dependent diabetes mellitus).

3. Gestational diabetes: Diabetes caused by pregnancy is called gestational diabetes. Sugar levels in a mother circulate through the placenta to the baby, gestational diabetes needs to be controlled to protect the baby's growth and development. The rate of gestational diabetes is between 2% and 10% in pregnancies.

4. Prediabetes: This type is the stage before t2dm your blood glucose levels are higher than normal but not high enough to diagnose type 2 diabetes.

Metformin Hydrochloride (MTF) (C4H12N5Cl) is 1; 1-dimethylbiguanidine monohydrochloride is an anti-diabetic drug from the biguanide class of oral hypoglycaemic agents & taken orally in the treatment of non-insulin dependent diabetes mellitus. Majorly taken metformin HCl will increase in glucose transport across the cell in skeletal muscle.

Sitagliptin phosphate monohydrate (SPM) (C16H20F6N5O6P) is (3R)-3-amino-1-[3-(trifluoromethyl)- 5,6-dihydro [1,2,4] triazolo [4,3-a] pyrazin-7(8H)-yl]-4-(2,4,5-trifluorophenyl) butan-1-one phosphate hydrate is oral hypoglycaemic drug of the dipeptidyl peptidase-4 (DPP-4) inhibitor class. This represents a new therapeutic view in treating of type 2 diabetes. This is done through inhibition of the inactivation of incretins, particularly glucagon like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP), thereby improving glycaemic control.

Literature survey also revealed several analytical methods such as simple and stability indicating UV, Spectro-fluorimetry, RP-HPLC, HPTLC and LC-MS/MS was reported for the determination of metformin hydrochloride.
Literature survey also revealed several analytical methods such as simple and stability indicating UV, Spectro-fluorimetry, RP-HPLC, LC-MS/MS was reported for the determination of sitagliptin phosphate monohydrate.\(^2\)

**Drug Profile**

- **Metformin Hydrochloride (MTF)**

<table>
<thead>
<tr>
<th>Structure</th>
<th><img src="image" alt="Structure" /></th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical name</td>
<td>N,N-dimethyl imido dicarbonimidic diamide hydrochloride</td>
</tr>
<tr>
<td>Molecular formula</td>
<td>C(<em>4)H(</em>{12})N(_5)Cl</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>165.62 g/mol</td>
</tr>
<tr>
<td>Appearance</td>
<td>White crystalline compound</td>
</tr>
<tr>
<td>Category</td>
<td>Biguanide</td>
</tr>
<tr>
<td>Melting point</td>
<td>223-242°C</td>
</tr>
<tr>
<td>Solubility</td>
<td>&gt;300 mg/mL in water</td>
</tr>
</tbody>
</table>

- **Sitagliptin Phosphate monohydrate (SPM)**

<table>
<thead>
<tr>
<th>Structure</th>
<th><img src="image" alt="Structure" /></th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical name</td>
<td>(3R)-3-amino-1-[3-(trifluoromethyl)-5,6-dihydro [1,2,4] triazolo[4,3-a]pyrazin-7(8H)-yl]-4-(2,4,5-trifluorophenyl) butan-1-one phosphate hydrate</td>
</tr>
<tr>
<td>Molecular formula</td>
<td>C(<em>{16})H(</em>{20})F(_6)N(_5)O(_6)P</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>523.32 g/mol</td>
</tr>
<tr>
<td>Appearance</td>
<td>White powder</td>
</tr>
<tr>
<td>Category</td>
<td>Dipeptidyl peptidase-4 (DPP-4) inhibitor</td>
</tr>
<tr>
<td>Melting point</td>
<td>206.37°C</td>
</tr>
<tr>
<td>Solubility</td>
<td>69.5 mg/dl in water / octanol at 24.5°C</td>
</tr>
</tbody>
</table>

**Mechanism of action**

While the mechanisms of action through which metformin exerts its various effects remains somewhat unclear, it is clear that metformin contributes to glycaemic control in a variety of ways. In a review of the available research in 1999, Wiernsperger NF, Bailey reported that metformin assists in glycaemic control in many ways including reducing hepatic glucose output, increasing peripheral glucose utilization, decreasing fatty acid oxidation, reducing appetite and weight gain, sensitizing peripheral tissue to insulin action increasing the functional activity of glucose transporters & increasing insulin mediated receptor tyrosine kinase range of insulin signals.\(^3\)
The accurate mechanism of action of metformin lowers the basal and postprandial blood glucose in T2DM are not completely undertaken. But, at least some of the therapeutic effects of metformin are determined through GLP-1.\(^4\) Metformin sometime increases GLP-1 level than an oral glucose increase weight in non-diabetic patients. And it has been suggested that metformin does not directly inhibit DPP-4 but rather it enhances GLP-1 secretion. Since metformin appears to increase levels of GLP-1 in a different way than the DPP-4 inhibitors the combined effect of metformin and sitagliptin is thought to be complimentary.

Insulin is secreted in response to elevation of plasma glucose. But it has been found that oral glucose intake augments insulin secretion 3 to 4 folds more as compared to an IV infusion of glucose that results in an identical elevation of plasma glucose. This augmentation of insulin secretion follows the inhale of oral glucose is known as the incretin effect.\(^5\)

It has been found that lower concentrations of active GLP-1 associated with an inadequate insulin response. Patients with T2DM have almost at greatly reduced incretin effect. The GLP-1 and GIP that are rapidly degraded and inactivated through metabolism mediated by the DPP-4 enzyme.\(^6\)

DPP-4 is a serine protease located on the surface of cells in the kidneys, intestines, bone marrow, liver, pancreas, placenta, thymus, spleen, epithelial cells, vascular endothelium, and lymphoid and myeloid cells.\(^7\) DDP-4 inhibitors such as sitagliptin block the enzymatic inactivation of the incretins which results in higher levels of active incretins in circulation.

Since the insulin releasing effects of the incretins are glucose dependent, insulin levels are only increased in response to the body’s need for insulin which decreases the patient’s risk of hypoglycaemic while still improving glycaemic control.\(^6,8\)

**Reported Analytical Methods**

<table>
<thead>
<tr>
<th>Sn</th>
<th>Title</th>
<th>Method</th>
<th>Description</th>
<th>Detection mode</th>
<th>Reference no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Method development and validation of sitagliptin and metformin using reverse phase HPLC method in bulk and tablet dosage form</td>
<td>reverse phase HPLC</td>
<td>Mobile phase: 0.1% potassium dihydrogen orthophosphate: methanol(50: 50 v/v) pH: 8.5 Flow rate: 1.0ml/min Retention time: 2.3 min &amp; 4.6 min</td>
<td>Detection wavelength at 215 nm</td>
<td>[9]</td>
</tr>
<tr>
<td>2.</td>
<td>Method development of simultaneous estimation</td>
<td>UV-visible</td>
<td>Linearity range: 20-60 µg/ml &amp; 2-10µg/ml Correlation</td>
<td>Wavelength at 267 nm &amp; 231 nm</td>
<td>[10]</td>
</tr>
<tr>
<td>Step</td>
<td>Methodology</td>
<td>Details</td>
<td></td>
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</tr>
<tr>
<td>1.</td>
<td>Spectrophotometric determination of sitagliptin and metformin</td>
<td>Coefficient: 0.999 &amp; 0.990 % Recovery: 98.1 &amp; 98.37% LOD: 0.954 &amp; 1.2 μg/ml LOQ: 2.89 &amp; 3.6 μg/ml</td>
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<tr>
<td>2.</td>
<td>Spectrophotometric method</td>
<td>Linearity range: 25-500 μg/ml Correlation Coefficient: 0.9997 &amp; 0.9999 Variance: 0.983 &amp; 2.143</td>
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<td>3.</td>
<td>Simultaneous determination of sitagliptin phosphate monohydrate &amp; metformin hydrochloride in tablets by a validated UPLC method.</td>
<td>Mobile phase: potassium dihydrogen phosphate: hexane-1-sulfonic salt (10mM:2mM) pH: 5.50 Flow rate: 0.2 ml/min LOD: 0.2 &amp; 0.06 μg/ml LOQ: 0.7 &amp; 0.2 μg/ml Wavelength at 210nm</td>
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<tr>
<td>4.</td>
<td>Development and validation of a method for simultaneous estimation of metformin and sitagliptin in human plasma by LC–MS-MS and Its application in a bioequivalence study.</td>
<td>Mobile phase: methanol :water (1 : 1, v/v) pH: 4.5 &amp; 0.2, Recovery: 92 &amp; 104.5 % Retention time: 3.20 min Flow rate: 0.2 ml/min, LOD: 2.5 &amp; 0.75 ng/ml LOQ: 92 &amp; 104.5 % Wavelength at 267 nm</td>
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<tr>
<td>5.</td>
<td>Development and validation of RP-HPLC method for determination of metformin and sitagliptin in bulk and pharmaceutical dosage form.</td>
<td>Mobile phase: water :methanol 60:40 v/v Retention time: 2.86 &amp; 3.94 min Flow rate: 1 ml/min Run time: 5 min LOD: 0.663 &amp; 0.405 μg/ml LOQ: 1.92 &amp; 1.228 μg/ml Linearity: 20-80 μg/ml Wavelength at 258 nm</td>
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<tr>
<td>6.</td>
<td>Development &amp; validation of RP-HPLC method for the analysis of metformin.</td>
<td>Mobile phase: methanol:water (30:70 v/v) Retention time: 4.4 min Flow rate: 0.5 ml/min LOD: 0.1 μg/ml LOQ: 0.3 μg/ml Linearity: 0.312-5 μg/ml Wavelength at 233 nm</td>
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<tr>
<td>7.</td>
<td>Development &amp; validation of RP-HPLC method for the estimation of sitagliptin phosphate in bulk and pharmaceutical dosage form.</td>
<td>Mobile phase: 0.01 M potassium dihydrogen phosphate : methanol (50:50 % v/v) pH: 2.5 Wavelength at 267 nm</td>
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<tr>
<td>8.</td>
<td>Development &amp; validation of RP-HPLC method for the estimation of sitagliptin phosphate in bulk and pharmaceutical dosage form.</td>
<td>Mobile phase: potassium dihydrogen phosphate: hexane-1-sulfonic salt (10mM:2mM) pH: 5.50 Flow rate: 0.2 ml/min LOD: 0.2 &amp; 0.06 μg/ml LOQ: 0.7 &amp; 0.2 μg/ml Wavelength at 210nm</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

[11] [12] [13] [14] [15] [16]
Flow rate: 1 ml/min  
Run time: 20 min  
LOD: 3 μg/ml  
LOQ: 10 μg/ml  
Linearity: 10-20 mg/ml  
Correlation Coefficient: 0.9998  
Recovery: 99-101 %  
Precision: 53.90 %  
Robustness: 20 mg/ml  
Ruggedness: 234_+ 5 nm | Wavelength at 266 nm | [17] |
| --- | --- | --- | --- | --- |
| 10. Development & validation of UV-spectrophotometric method for estimation of metformin in bulk & tablet dosage form. | UV-spectrophotometric method | Linearity: 10-20 mg/ml  
Correlation Coefficient: 0.9998  
Recovery: 99-101 %  
Precision: 53.90 %  
Robustness: 20 mg/ml  
Ruggedness: 234_+ 5 nm | Wavelength at 234 nm | [18] |
| 11. Development & validation of stability indicating UV spectrophotometric method for the estimation of sitagliptin phosphate in bulk & tablet dosage form. | UV spectrophotometric method | Linearity: 10-100 μg/ml  
Accuracy: 99.87-100.45 %  
LOD: 0.16 μg/ml  
LOQ: 0.45 μg/ml  
Precision: 1.3-1.29 % | Wavelength at 200-267 nm | [19] |

**CONCLUSION**

The proposed method for the assay precision, linearity, LOD, LOQ of the popular antidiabetic drugs. Method validation of metformin and sitagliptin was commercially, economically and rapidly studied in tablet dosage form. It can be easily adopted for routine QC monitoring of the API, in-process samples, finished products etc.

**REFERENCES**


20.