DEVELOPMENT & VALIDATION OF STABILITY INDICATING HPLC METHOD FOR DETERMINATION OF SOLIFENACIN IN BULK FORMULATIONS

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ABSTRACT
A stability indicating, isocratic, rapid, specific, and sensitive high performance liquid chromatographic (HPLC) method was developed and validated for determination of Solifenacin in bulk drug. The quantitative determination of SOLIFENACIN was performed on a Hypersil C18 (250mm × 4.6 mm id., 5 µm) column with mobile phase consist of 10 mM potassium dihydrogen phosphate buffer with 0.1% v/v TEA in Milli-Q water (pH=3): Acetonitrile (60:40 %v/v) pumped at a flow rate of 1 mL min⁻¹ at 25 °C. The eluents were monitored at wavelength 210 nm using photo diode array (PDA) detector. The drug was subjected oxidation, hydrolysis and heat to apply stress conditions. The developed method was validated as per ICH guidelines for the parameters such as specificity, linearity, precision, accuracy, limit of detection, limit of quantification and found to be satisfactorily. The response was linear in the drug concentration range of 1–30 µg mL⁻¹ (r²=0.9991) with limit of detection and limit of quantification 0.019 µg mL⁻¹ and 0.063 µg mL⁻¹ respectively. The Relative Standard Deviation (RSD) values for intra- and inter-day precision studies were 0.50% and 0.62% respectively. Mean % recovery (mean ± SD) was found to be 100.51 ± 1.98.

Keywords: Solifenacin, Stability Indicating, HPLC, Photo diode array detector

INTRODUCTION
Solifenacin is a prescription drug used for the treatment of overactive bladder in adults with symptoms of urinary incontinence, urinary urgency and urinary frequency.

The chemical name of Solifenacin succinate is butanedioic acid, compound with (1S)-(3R)-1-azabicyclo [2, 2, 2] oct-3-yl 3, 4-dihydro-1-phenyl-2(1H)-isoquinoline carboxylte (1:1) and the empirical formula is C₂₃H₂₆N₂O₂.C₄H₆O₄ and its molecular weight is 480.55 and structural formula of Solifenacin succinate is represented in figure 1.

Fig. 1: Chemical structure of Solifenacin

Solifenacin is a competitive muscarinic receptor antagonist with selectivity for the urinary bladder over salivary glands. Solifenacin inhibits carbachol-induced intracellular calcium mobilization more potently in bladder smooth muscle cells than in salivary gland cells.

Solifenacin is approximately 98% (in vivo) bound to human plasma proteins, principally to α1-acid glycoprotein. Solifenacin is highly distributed to non-ICs tissues, having a mean steady-state volume of distribution 600 L.

Solifenacin is extensively metabolized in the liver by CYP3A4 isoenzyme; however, an alternative metabolic pathway is also reported. The primary metabolic routes of Solifenacin are through N-oxidation of the quinuclidin ring and 4R hydroxylation of tetrahydro isoquinoline ring. One pharmacologically active metabolite (4Rhydroxy Solifenacin), occurring at low concentrations and unlikely to contribute significantly to clinical activity, and three pharmacologically inactive metabolites (N-glucuronide and the N-oxide and 4R-hydroxy-N-oxide of Solifenacin) have been found in human plasma after oral dosing.

The major metabolites identified in urine were N-oxide of Solifenacin, 4R-hydroxy Solifenacin and 4R-hydroxy-N-oxide of Solifenacin, and in feces 4R-hydroxy Solifenacin. The elimination half-life of Solifenacin following chronic dosing is approximately 45 - 68 hour.

Solifenacin exerts greater selectivity for urinary bladder over salivary gland than tolterodine, oxybutynin, darifenacin and atropine, and may consequently provide symptomatic benefit in the treatment of overactive bladder with less dry mouth than currently used anti-muscarinic drugs.

Solifenacin succinate is a novel muscarinic receptor antagonist, approved for the treatment of overactive bladder with affinity for muscarinic M₃ receptor subtype, high degree of selectivity and to the fact that most tissues or organs express multiple muscarinic receptors.

M₁ and M₃ receptors are mainly distributed in the bladder while M₂ subtype is distributed predominantly in the salivary gland and that M₃ subtype plays a major role in the physiological function of both organs. Solifenacin compared with oxybutynin binds to a greater extent to bladder M₃ muscarinic receptors in the bladder while it may exert a relatively little activity to bind exocrine M₁ muscarinic receptors.

Various methods are available for the analysis of Solifenacin in literature like LC–ESI‐MS/MS, semi‐micro high performance liquid chromatography. Analytical method for the estimation of Solifenacin in bulk drug was not reported by HPLC method or HPTLC method.

The aim of this work was to develop an accurate, specific, precise stability-indicating HPTLC method for the determination of Solifenacin. The developed method was validated as per the International Conference on Harmonization (ICH) guideline.

Stability testing of new drug substances and products requires the stress testing to carry out and to elucidate the inherent stability characteristics of the active substance.

EXPERIMENT
Materials and methods
Solifenacin succinate (99.1 % as on dried basis) standard was kindly supplied by Cadila health care Ltd, Ahmedabad, India. HPLC grade methanol and acetonitrile were purchased from Merck chemicals,
Mumbai. The water for HPLC was prepared by double glass distillation and filtered through Milli Q water purification system. Potassium dihydrogen phosphate and orthophosphoric acid (OPA) were obtained from SD fine chemical Ltd. Mumbai, India.

HPLC consists of Shimadzu LC-10 ADVP solvent delivery system, SIL-10A DVP auto-injector, CTO-10A System oven, SPD-M10A VP photo diode array detector, SCL-10AVP System controller. The analytical column used was Hypersil C8 column (250 mm × 4.6 mm i. d., 5 µm particle size). All analysis was carried out at a temperature of 25°C under isocratic conditions. The mobile phase consisted of a mixture of 10 mM potassium dihydrogen phosphate buffer with 0.1 % TEA in pH 3 (pH 3, adjusted with OPA): acetonitrile (60:40, v/v). The flow rate was 1 mL min−1; the volume of injection was 20 µL, total run time was 12 min, and the detection was made at 210 nm. The mobile phase was filtered through Millipore filter (0.45 µm) and was degassed prior to use. All data integration was performed using class VP software.

**Standard preparation**

The assay concentration of Solifenacin was selected as 10 µg mL−1. It was prepared by weighing 10 mg of Solifenacin and it was dissolved in 10 ml methanol to get concentration of 1 mg mL−1 of Solifenacin. It was diluted with methanol to get a concentration of 100 µg mL−1 (working stock). Further dilutions were carried out using the mobile phase (acetonitrile: 10 mM potassium dihydrogen phosphate buffer (pH 3) in the ratio of 40:60) to get resultant concentration of 10 µg mL−1.

**Linearity**

From the working stock solution (100 µg mL−1), the serial dilutions were done to get the concentration of 1, 5, 10, 15, 20, 25, and 30 µg mL−1 respectively. A volume of 20 µL from each concentration of the solution was injected and chromatograms were recorded under the optimized chromatographic conditions. The peak area of the drug was plotted against the concentration of the drug.

**Repeatability**

Repeatability is the result of the method operating over a short time interval (within a day) under the same conditions. The peak area of assay concentration of the drug (10µg mL⁻¹) was analyzed six times on the same day. The percentage relative standard deviation (% RSD) was calculated for the resultant peak area.

**Intermediate precision**

To assess the degree of reproducibility of the method, assay conc. (10µg mL⁻¹) was analyzed on different day. The assay procedure was repeated six times and the chromatogram was recorded and the % RSD was calculated.

**Accuracy**

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as conventional true value or accepted reference value and the value found. The accuracy was done by recovery studies. The known amount of standard drug was spiked to the pre-analyzed samples and the recovery of the drug was calculated. Accuracy was performed at 3 levels of 80, 100 and 120% of standard concentration. A solution containing 10 µg mL−1 of the sample was spiked with 80, 100, and 120% of the standard Solifenacin solution (8, 10, 12 µg mL−1) and analyzed in same chromatographic condition. The percentage recovery was calculated from the peak area of the drug.

**Robustness**

It is the capacity of a method to remain unaffected by small deliberate variations in method parameters:

- Influence of variations in flow rate (±0.1 mL min−1)
- Influence of variations of pH of mobile phase (±0.2)
- Influence of change in temperature (±5°C)

**Influence of variations in composition of mobile phase (±2%)**

**Limit of Detection (LOD) and Limit of Quantification (LOQ)**

LOD is ability of analytical method to detect the lowest conc. of the analyte. LOQ is the lowest conc. of the analyte, which can be quantitatively analyzed with acceptable precision and accuracy. It can be calculated based on signal to noise ratio.

**Assay of formulation**

The tablets were purchased from a local drug store (Solifenacin 10 mg Tab) supplied by Ranbaxy pharmaceuticals, India. 10 Tablets were weighed and average weight was calculated. An amount equivalent to 10 mg of Solifenacin was weighed and dissolved in methanol. Then the solution was sonicated for 30 min and filtered. The filtrate was further diluted to get a concentration of 10 µg mL−1 (theoretical value) and was injected to the HPLC system.

**System suitability parameters**

These parameters include plate number (N), capacity factor (K'), asymmetry factor, and relative standard deviation (RSD) for peak area for repetitive injections. These parameters were within the acceptable limits.

**Stock solution stability**

The freshly prepared standard stock solution of Solifenacin 10 µg mL−1 was stored in the refrigerated temperature. The stability of the stock was analyzed after one month duration and compared with the initial concentration.

**Specificity**

Specificity was established by determination of purity of the drug peak using a PDA detector.

**Degradation studies**

**Alkali hydrolysis**

25 mg of Solifenacin was weighed accurately and transferred to 25 mL volumetric flask and dissolved in 1 M sodium hydroxide solution. Immediately after making up the volume, the solution was transferred to round bottom flask and kept for refluxing at 80 °C, 1 mL sample was taken at 48 hrs and the sample was neutralized with 1 M hydrochloric acid solution. It was further diluted to 10 mL with mobile phase and loaded into HPLC system.

**Acid hydrolysis**

25 mg of Solifenacin was weighed accurately and transferred to 25 mL volumetric flask and dissolved in 1 M hydrochloric acid solution. Immediately after making up the volume, the solution was transferred to round bottom flask and kept for refluxing at 80 °C, 1 mL sample was taken at 48 hrs and the sample was neutralized with 1 M sodium hydroxide solution and diluted to 10 mL with mobile phase and loaded into HPLC system.

**Oxidative degradation**

25 mg of Solifenacin was weighed accurately and transferred to 25 mL volumetric flask and dissolved in 3% v/v hydrogen peroxide solution. Immediately after making up the volume, the solution was transferred to round bottom flask and kept at room temperature, 1 mL samples was withdrawn at 48 hrs and diluted to 10 mL with mobile phase and loaded into HPLC system.

**Thermal degradation**

Powdered API was kept in a hot air oven at 65°C for 3 days. The drug was found to be stable to dry heat.

**RESULTS AND DISCUSSION**

**Method development**

The chromatographic conditions were adjusted in order to provide a good assay performance. Initial trials were done on Supelco C18 column (250 mm × 4.6 mm i. d., 5 µm), but the peak was distorted. Finally, Hypersil C8 column (250 mm × 4.6 mm i. d., 5 µm particle
size) was selected. Optimization of mobile phase was performed based on peak parameters (resolution of drugs and degradation products, tailing), sensitivity of the assay, ease of preparation, readily available, and run time. The mobile phase consisted of a mixture of 10 mM potassium dihydrogen phosphate buffer with 0.1% TEA v/v at pH 3 (pH 3, adjusted with OPA): acetonitrile (60:40, v/v) was selected which gave sharp and very well resolved peaks. The volume of injection was 20 µL and detection was made at 210 nm. The retention time for Solifenacin was 7.82 min and the total run time was 12 min (Fig.2).

**Fig. 2: Chromatogram of Solifenacin standard**

### Robustness

The robustness of assay method was studied by incorporating small but deliberate changes in the analytical method (e.g. variations in flow rate, column temperature, mobile phase composition, pH of buffer, etc.) and also by observing the stability of the drugs for 24 hr at room temperature in the dilution solvent. In all the varied chromatographic conditions, there was no significant change in chromatographic parameters. Result is given in table 3.

**Table 3: Robustness of the method (n=6).**

<table>
<thead>
<tr>
<th>Condition changed</th>
<th>SD of mean peak area</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase composition</td>
<td>2836</td>
<td>0.47</td>
</tr>
<tr>
<td>pH of mobile phase</td>
<td>2199</td>
<td>0.30</td>
</tr>
<tr>
<td>Temperature</td>
<td>2514</td>
<td>0.64</td>
</tr>
<tr>
<td>Flow</td>
<td>3288</td>
<td>0.47</td>
</tr>
</tbody>
</table>

### Assay of marketed formulation

Solifenacin R value of 7.89 min was observed for the chromatogram obtained from the drug samples extracted from the tablets formulation. There was no interference from excipients. The assay of Solifenacin was found to be 98.79%.

### Alkali hydrolysis

Initially 0.5 M Sodium hydroxide concentration was used for the degradation of Solifenacin but it was found that the drug was stable and there was no degradation. So 1 M sodium hydroxide was used further and it showed that drug was degraded in 1 M sodium hydroxide. The degraded peak was found at the retention time of 4.3 minutes. The spectrum of the degradation product was taken and found that spectra's were matching to the standard Solifenacin spectra. Peak purity index (0.9999) was greater than peak purity threshold (0.9991) which indicates that there is no merging of impurity peaks and peak is pure (Fig.3, 4, 5).

**Fig. 3: Chromatogram of Solifenacin in 1N sodium hydroxide at 80 °C**
Acid induced degradation

Initially 0.5 M Hydrochloric acid concentration was used for the degradation of Solifenacin but it was found that the drug was stable and there was no degradation. So 1M hydrochloric acid was used further and it showed that the drug degraded in 1M hydrochloric Acid. The degraded peak was at the retention time of 4.3 minutes. The spectrum of the degradation product was taken and found that spectra's were matching to that of standard Solifenacin spectra. Peak purity index (0.9999) was greater than peak purity threshold (0.9991) which indicated that there is no merging of impurity peaks and peak is pure(fig. 6, 7, 8).

Oxidative degradation

Initially 3% hydrogen peroxide was used for the degradation of Solifenacin, it showed that drug was degraded in 3% hydrogen peroxide. The degraded peak was found at the retention time of 5.3 minutes. The spectrum of the degradation product was taken and found that spectra's were matching to the standard Solifenacin spectra. Peak purity index (0.9999) was greater than peak purity threshold (0.9991) which indicated that there is no merging of impurity peaks and peak is pure(fig.9, 10, 11).

Thermal degradation

There was no degradation observed at 65 °C for 2 weeks. It indicated that the drug is stable to heat.

Degradation results are given table 4.
### Table 4: Thermal degradation of the product

<table>
<thead>
<tr>
<th>Stress condition applied</th>
<th>Retention times of degradation products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaline hydrolysis</td>
<td>4.36 min.</td>
</tr>
<tr>
<td>Acid hydrolysis</td>
<td>4.35 min.</td>
</tr>
<tr>
<td>Oxidative degradation</td>
<td>5.10 min.</td>
</tr>
</tbody>
</table>

### Table 5: Summary of validation data

<table>
<thead>
<tr>
<th>Validation property</th>
<th>Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range (µg/mL)</td>
<td>1-30</td>
</tr>
<tr>
<td>Coefficient of determination</td>
<td>0.9991 ± 0.01</td>
</tr>
<tr>
<td>Limit of detection (µg/mL)</td>
<td>0.019</td>
</tr>
<tr>
<td>Limit of quantification (µg/mL)</td>
<td>0.063</td>
</tr>
<tr>
<td>% Recovery (n = 6)</td>
<td>100.51</td>
</tr>
<tr>
<td>Precision [RSD [%]]</td>
<td></td>
</tr>
<tr>
<td>Intra-day precision (n = 6)</td>
<td>0.50</td>
</tr>
<tr>
<td>Intermediate precision(n=6)</td>
<td>0.62</td>
</tr>
</tbody>
</table>

### CONCLUSION

This study is a typical example of development of a stability-indicating assay, established following the recommendations of ICH guidelines. It is one of the studies where forced decomposition studies were done under all different suggested conditions and the products were resolved in a single isocratic run. In literature, most of the studies on development of stability-indicating assays involve either forced decomposition studies under only one or two conditions or separation of drug from major degradation products whose standards were available. The developed method is simple, accurate, precise, specific, selective and rugged. It is proposed for analysis of the drug and degradation products in stability samples in industry.

### REFERENCES

7. ICH Q2 (R1) Validation of Analytical Procedures, Text and Methodology, 2005.