ABSTRACT

Japanese Pharmacopoeia

Sodium is official in Japanese Pharmacopoeia as a piperidine derivative, 2RS)-2-Methyl-1-(4-methylphenyl)-3-piperidin-1-yl propan-1-one monohydrochloride (Fig 1a), and TOL is chemically 2RS)-2-Methyl-1-(4-methylphenyl)-3-dichlorophenyl)-amino[phenyl acetate (Fig 1b), used as an analgesic and anti-inflammatory drug used in the treatment of rheumatoid arthritis, osteoarthritis and alkylosing spondylitis and also for a variety of non-rheumatic inflammatory conditions.

INTRODUCTION

TOL is chemically 2RS)-2-Methyl-1-(4-methylphenyl)-3-piperidin-1-yl propan-1-one monohydrochloride (Fig 1a), a piperidine derivative centrally acting muscle relaxant which is used in the treatment of different pathological conditions like acute and chronic muscle spasm, electroconvulsive therapy, neurological conditions and orthopedic manipulation, myelopathy, encephalomyelitis, spondyloysis, spondylarthrosis, cervical and lumbar syndrome, arthritis of the large joints obliterating anghrothromboangiitis obliterans, raynaud's syndrome. TOL is official in Japanese Pharmacopoeia. Chemically DFS is, sodium 2-[(2,6-dichlorophenyl)-amino]phenyl acetate (Fig 1b), used as analgesic and anti-inflammatory drug used in the treatment of rheumatoid arthritis, osteoarthritis and alkylosing spondylitis and also for a variety of non-rheumatic inflammatory conditions. Didclofenac sodium is official in Japanese Pharmacopoeia. British Pharmacopoeia, United States Pharmacopoeia and Indian Pharmacopoeia.

The review of literature revealed that various analytical methods involving spectrophotometry, HPLC, HPTLC have been reported for TOL in single form and in combination with other drugs. Several analytical methods have been reported for DFS in single form and in combination with other drugs including spectrophotometry, HPLC, LC-MS, HPTLC.

To the best of our knowledge, there is no published spectrophotometric method for this combination. So, the present paper describes a simple, accurate and precise method for simultaneous estimation of TOL and DFS in their combined tablet dosage form.

METHOD FOR SIMULTANEOUS ESTIMATION OF TOLPERISONE HYDROCHLORIDE AND DICLOFENAC SODIUM IN THEIR COMBINED TABLET DOSAGE FORM

DEVELOPMENT AND VALIDATION OF SIMULTANEOUS EQUATION SPECTROPHOTOMETRIC METHOD

A simple, accurate, precise and specific spectrophotometric method has been developed for simultaneous determination of Tolperisone Hydrochloride (TOL) and Diclofenac Sodium (DFS) in its combined tablet dosage form by using methanol as a solvent. The method involves solving of simultaneous equation based on measurement of absorbance at two wavelengths at 254 nm and 282 nm. Method follows Beer's linearity in the range of 5-35μg/ml for TOL and DFS both. The mean % recoveries were found to be in the range of 99.35 – 100.40% and 98.70 – 100.20 % for TOL and DFS respectively. Limit of Detection and quantitation was found to be 0.101µg/ml and 0.306µg/ml for TOL and 0.120µg/ml and 0.364µg/ml for DFS respectively. Assay results of market formulation were found to be 99.70 and 99.40 % for TOL and DFS respectively. The proposed method has been validated as per ICH guidelines and successfully applied to the estimation of TOL and DFS in their combined Tablet dosage form.

Keywords: Tolperisone Hydrochloride, Diclofenac Sodium, Simultaneous Equation Method, Analytical Method validation.

MATERIALS AND METHODS

Reagents and chemicals

Analytically pure TOL and DFS were kindly provided by Zydus Cadila Healthcare Ltd, Ahmedabad, Gujarat, India and Medico labs, Ahmedabad, Gujarat, India respectively as gratis samples. Analytical grade methanol was purchased from RFCL limited, New Delhi, India. Tablet of TOL and DFS in combined dosage form, TOLP-IDOL-D, was procured from local market.

Fig. 1: Chemical structure of (a) TOL and (b) DFS

Instruments

Two spectrophotometers were used for study, a Shimadzu UV/Vis 1800 double beam spectrophotometer with a wavelength accuracy (± 0.3 nm), 1 cm matched quartz cells and UV probe 232 software was used for all the spectral measurements and Shimadzu UV/Vis 1601 double beam spectrophotometer with a wavelength accuracy (± 0.3 nm) and 1 cm matched quartz cells was used for reproducibility study. Calibrated analytical balance KEA 210 (K-Roy Instrument Pvt. Ltd) was used for weighing purpose.
Preparation standard stock solutions
Accurately weighed 100 mg of TOL and DFS standard were transferred to a separate 100 ml volumetric flask and dissolved in 50 ml methanol. The flasks were shaken and volume was made up to the mark with methanol to give solutions containing 1000 µg/ml TOL and 1000 µg/ml DFS. From this solution 10 ml was transferred to volumetric flask of 100 ml capacity. Volume was made up to the mark to give a solution containing 100µg/ml of TOL and 100µg/ml DFS.

**Simultaneous equation method**
5-35 µg/ml solutions of TOL and DFS were prepared in methanol by appropriate dilution and spectrum was recorded between 200-400 nm. This method of analysis was based on the absorption of drugs TOL and DFS at the wavelength maxima of each other. Two wavelengths were selected for the development of the simultaneous equations at 254 nm and 282 nm (figure 2). The absorptivity values E (1%, 1 cm) were determined for two drugs at all selected wavelengths. The concentration of two drugs in mixture was calculated by using following equations.

\[
C_x = \frac{(A_x - A_{x2})}{(A_{y1} - A_{y2})}
\]

\[
C_y = \frac{(A_y - A_{y2})}{(A_{x1} - A_{x2})}
\]

Where, \( C_x \) and \( C_y \) are the concentrations of TOL and DFS respectively in mixture and in sample solutions. \( A_x \) and \( A_y \) are the absorbances of sample at 254 nm and 282 nm, respectively, \( A_{x1} \) and \( A_{x2} \) are the absorbivity of TOL at 254 nm and 282 nm, respectively. \( A_{y1} \) and \( A_{y2} \) are the absorbivity of DFS at 254 nm and 282 nm. All standard and sample solutions absorbance was measured at 254 nm and 282 nm with their respective blanks.

**Method validation**
The proposed method has been extensively validated in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), robustness and reproducibility. The accuracy was expressed in terms of percent recovery of the known amount of the standard drugs added to the known amount of the pharmaceutical dosage forms. The precision (Coefficient of Variation - CV) was expressed with respect to the repeatability, intra-day and inter-day variation in the expected drug concentrations. After validation, the developed method has been applied to pharmaceutical dosage form.

**Specificity**
Commonly used excipients (starch, microcrystalline cellulose and magnesium stearate) were spiked into a pre weighed quantity of placebo which were sonicated for 15 min. The overlain spectra of TOL and DFS reveals that the both the drugs exhibits distinct \( \lambda_{max} \) and also both drugs shows absorbance at the \( \lambda_{max} \) of each other. For estimation of TOL and DFS using spectrophotometry simultaneous equation method was decided to be used. In this method two wavelengths are required. One wavelength is selected at which TOL shows maximum absorbance (254 nm), while second wavelength is selected at which DFS shows maximum absorbance (282 nm).

**Detection limit and Quantitation limit**
ICH guideline describes several approaches to determine the detection and quantitation limits. These include visual evaluation of signal-to-noise ratio and the use of standard deviation of the response and the slope of the calibration curve. In the present study, the LOD and LOQ were based on the third approach and were calculated according to the 3σ/s and 10σ/s criterions, respectively; where σ is the standard deviation of y-intercepts of regression lines and s is the slope of the calibration curve.

**Robustness**
The sample solution was prepared and then analyzed with change in the typical analytical conditions like stability of analytical solution.

**Reproducibility**
The absorbance readings were measured at different laboratory for sample solution using another spectrophotometer by analyst and the values obtained were evaluated using t-test to verify their reproducibility.

**Determination of TOL and DFS in their Combined Dosage**
Twenty tablets were weighed and powdered. A powder quantity equivalent to 150 mg TOL and 50 mg DFS was accurately weighed and transferred to volumetric flask of 100 ml capacity. 60 ml of methanol was transferred to this volumetric flask and sonicated for 15 min. The above solution was filtered through whatman filter paper (0.45µ). The filtrate was diluted with methanol to give solutions containing 100 µg/ml of TOL and 100 µg/ml of DFS. The resulting solution was analysed by proposed methods. The quantitation was carried out by keeping these values to the straight line equation of calibration curve.

**RESULTS AND DISCUSSION**

**Simultaneous equation Spectrophotometric method for TOL and DFS combined dosage form- tablet**
Owing to the solubility of TOL and DFS in methanol it was selected as solvent. From overlain spectra of TOL and DFS it is clear that TOL exhibits \( \lambda_{max} \) at 254 nm and DFS exhibits \( \lambda_{max} \) at 282 nm. The overlain spectra of TOL and DFS reveals that the both the drugs exhibits distinct \( \lambda_{max} \) and also both drugs shows absorbance at the \( \lambda_{max} \) of each other. Estimation of TOL and DFS using spectrophotometry simultaneous equation method was decided to be used. In this method two wavelengths are required. One wavelength is selected at which TOL shows maximum absorbance (254 nm), while second wavelength is selected at which DFS shows maximum absorbance (282 nm).

**Simultaneous equation generated:**
\[
C_x = \frac{(A_{x1} \times 0.0112 - A_{x2} \times 0.00374)}{(0.0071 \times 0.0112 - 0.0502 \times 0.00374)}
\]

Where,
1) \( A_1 \) and \( A_2 \) is absorbance of sample at 254 nm and 282 nm respectively
2) \( C_x \) is concentration of TOL in µg/ml

\[
C_y = \frac{(A_{y1} \times 0.0071 - A_{y2} \times 0.0502)}{(0.0071 \times 0.0112 - 0.0502 \times 0.00374)}
\]

Where,
1) \( A_1 \) and \( A_2 \) is absorbance of sample at 254 nm and 282 nm respectively
2) \( C_y \) is concentration of DFS in µg/ml.

The % recoveries were found to be in the range of 99.35 – 100.4% for TOL and 98.70 – 100.20% for DFS (Table 4). The precision of method was determined by repeatability, intraday and interday
precision and was expressed as the C.V. (Table 1) which indicates good method precision.

The Limit of detection for TOL and DFS was found to be 0.101µg/ml and 0.120µg/ml respectively. Limit of quantification for TOL and DFS was found to be 0.306µg/ml and 0.364µg/ml at 254 nm and at 282 nm respectively (Table 2-3).

The methods was found to be specific, as there was no interference observed when the drugs were estimated in presence of excipients and robust, as there was no significant change in absorbance up to 24 hours of preparation of solution in methanol. The proposed spectrophotometric method was successfully applied to TOL and DFS combined dosage form.

Fig. 2: Overlaid spectrum of TOL (30µg/ml) and DFS (10µg/ml) in methanol

Calibration curves for TOL and DFS were plotted between absorbance and concentration (Fig. 3, 4). The following equations for straight line were obtained for TOL and DFS.

**Calibration Curve of TOL at 254 nm**

\[ y = 0.0502x - 0.0301 \]

\[ r^2 = 0.9994 \]

**Calibration Curve of TOL at 282 nm**

\[ y = 0.0071x + 0.0126 \]

\[ r^2 = 0.9988 \]

**Calibration Curve of DFS at 254 nm**

\[ y = 0.0112x + 0.0164 \]

\[ r^2 = 0.9972 \]

**Calibration Curve of DFS at 282 nm**

\[ y = 0.0374x + 0.0981 \]

\[ r^2 = 0.9972 \]

**Table 1: Summary of Validation Parameters of simultaneous equation method**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (µg/ml)</th>
<th>Linear Equation</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOL</td>
<td>0-40</td>
<td>Y = 0.0502x - 0.0301</td>
<td>0.9994</td>
</tr>
<tr>
<td>DFS</td>
<td>0-40</td>
<td>Y = 0.0071x + 0.0126</td>
<td>0.9988</td>
</tr>
<tr>
<td>TOL</td>
<td>0-40</td>
<td>Y = 0.0112x + 0.0164</td>
<td>0.9972</td>
</tr>
<tr>
<td>DFS</td>
<td>0-40</td>
<td>Y = 0.0374x + 0.0981</td>
<td>0.9972</td>
</tr>
<tr>
<td>Parameters</td>
<td>TOL</td>
<td>DFS</td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>-----</td>
<td>-----</td>
<td></td>
</tr>
<tr>
<td>Recovery %</td>
<td>99.35 – 100.4</td>
<td>98.70 – 100.20</td>
<td></td>
</tr>
<tr>
<td>Repeatability (C.V.) (n=6)</td>
<td>0.37</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>Precision</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intra-day (n=3)</td>
<td>0.19 – 0.97</td>
<td>0.18 – 0.50</td>
<td></td>
</tr>
<tr>
<td>Inter-day (n=3)</td>
<td>0.25 – 0.96</td>
<td>0.17 – 0.29</td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td>Specific</td>
<td>Specific</td>
<td></td>
</tr>
<tr>
<td>Robustness</td>
<td>Robust</td>
<td>Robust</td>
<td></td>
</tr>
<tr>
<td>Solvent suitability</td>
<td>Suitable for 24 hrs.</td>
<td>Suitable for 24 hrs.</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2: Statistical data TOL by Simultaneous Equation method

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TOL (at 254 nm)</th>
<th>TOL (at 282 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>5-35 µg/ml</td>
<td>5-35 µg/ml</td>
</tr>
<tr>
<td>Slope</td>
<td>0.0502x</td>
<td>0.0071x</td>
</tr>
<tr>
<td>Intercept</td>
<td>-0.030217</td>
<td>0.01267</td>
</tr>
<tr>
<td>Regression Coefficient (r²)</td>
<td>0.9994</td>
<td>0.9988</td>
</tr>
<tr>
<td>Standard deviation of Slope</td>
<td>0.000547</td>
<td>0</td>
</tr>
<tr>
<td>Standard deviation of Intercept</td>
<td>0.001538</td>
<td>0.000368</td>
</tr>
<tr>
<td>Limit of Detection (µg/ml)</td>
<td>0.101</td>
<td>0.171</td>
</tr>
<tr>
<td>Limit of Quantitation (µg/ml)</td>
<td>0.306</td>
<td>0.518</td>
</tr>
</tbody>
</table>

### Table 3: Statistical data DFS by Simultaneous Equation method

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DFS (at 254 nm)</th>
<th>DFS (at 282 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>5-35 µg/ml</td>
<td>5-35 µg/ml</td>
</tr>
<tr>
<td>Slope</td>
<td>0.0112x</td>
<td>0.0374x</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.016733</td>
<td>0.01267</td>
</tr>
<tr>
<td>Regression Coefficient (r²)</td>
<td>0.9972</td>
<td>0.9969</td>
</tr>
<tr>
<td>Standard deviation of Slope</td>
<td>0</td>
<td>0.0000816</td>
</tr>
<tr>
<td>Standard deviation of Intercept</td>
<td>0.000309</td>
<td>0.001364</td>
</tr>
<tr>
<td>Limit of Detection (µg/ml)</td>
<td>0.091</td>
<td>0.120</td>
</tr>
<tr>
<td>Limit of Quantitation (µg/ml)</td>
<td>0.276</td>
<td>0.364</td>
</tr>
</tbody>
</table>

### Table 4: Accuracy data for TOL and DFS by Simultaneous equation method

<table>
<thead>
<tr>
<th>% Level</th>
<th>Amount of drug added (µg/ml)</th>
<th>Amount recovered (µg/ml)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TOL (µg/ml)</td>
<td>DFS (µg/ml)</td>
<td>TOL (µg/ml)</td>
</tr>
<tr>
<td>50 %</td>
<td>10</td>
<td>10</td>
<td>9.96</td>
</tr>
<tr>
<td>100 %</td>
<td>20</td>
<td>20</td>
<td>19.87</td>
</tr>
<tr>
<td>150 %</td>
<td>30</td>
<td>30</td>
<td>30.12</td>
</tr>
</tbody>
</table>

### Table 5: Assay Results of Marketed Formulation

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Drug</th>
<th>Amount Taken (µg/ml)</th>
<th>Amount Found (µg/ml) (n = 3)</th>
<th>Labeled claim (mg)</th>
<th>Amount found per Tablet (mg)</th>
<th>% Label claim ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOLPIDOL-D</td>
<td>TOL</td>
<td>30</td>
<td>29.91</td>
<td>150</td>
<td>149.58</td>
<td>99.70 ± 0.2946</td>
</tr>
<tr>
<td>(Tablet)</td>
<td>DFS</td>
<td>10</td>
<td>9.94</td>
<td>50</td>
<td>49.70</td>
<td>99.40 ± 0.1743</td>
</tr>
</tbody>
</table>

### CONCLUSION

The proposed Simultaneous equation method provides simple, specific, precise, accurate and reproducible quantitative analysis for simultaneous determination of TOL and DFS in combined dosage form. The method was validated as per ICH guidelines in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), robustness and reproducibility. The proposed method can be used for routine analysis and quality control assay of TOL and DFS in combined dosage form.

### ACKNOWLEDGEMENT

Authors are thankful to Zydus Cadila Healthcare Ltd. (Ahmedabad, India) and Medico labs (Ahmedabad, India) for providing gratis sample with the great pleasure. The authors also thankful to Indubhai Patel College of Pharmacy and Research Centre (Dharmaj, India) for providing the necessary facilities for research work and to all the staff members and friends for their guidance and help throughout the research work.

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