**In vitro** dissolution profile comparison of an anti-migraine combinational drug in dosage form

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**Abstract** A novel **in vitro** dissolution profile was developed for formulated drug in combinational form containing naproxen sodium [NAP] and sumatriptan succinate [SUMA]. This study was performed to understand the dissolution of the drug in the physiological temperature and pH. Dissolution testing was performed using USP 29 type II testing apparatus rotating at 50 r/min in 900 mL deaerated buffer pH 1.2, 4.5 and 6.8 which was maintained at 37 ± 0.5 °C. Quantification was performed using a developed and validated high performance liquid chromatographic [HPLC] method. Aceclofenac [ACE] was used as internal standard. SUMA, ACE and NAP were eluted at 4.8, 5.7 and 7.9 min respectively. As expected for enteric coated immediate release [IR] tablets the dissolution of NAP and SUMA was rapid and essentially complete within 2 h using phosphate buffer pH 6.8. The comparison of the dissolution profiles was realized by model independent approach using a difference factor [f1], similarity factor [f2] and dissolution efficiency [DE]. Statistical results showed the profiles were similar to the reference and the test products. Hence this method demonstrated to be adequate for **in vitro** studies of NAP and SUMA in the combinational dosage form since there is no official monograph collaborating to the official codes.

**Key words** **in vitro** dissolution profiles, dissolution method development, naproxen sodium, sumatriptan succinate

**CLC number** O658 **Document code** A **Article IC** 1000-8712 201001-0093-07

In recent years considerable emphasis has been given to dissolution testing by the pharmaceutical industry and by regulatory authorities. The dissolution tests for immediate release [IR] solid oral dosage forms such as tablets are used to assess lot-to-lot quality of drug products guide development of new formulations and ensure continuing product quality and performance after certain changes made in formulation manufacturing process etc. 1. From a quality assurance point of view a more discriminating dissolution method is preferred because the test will indicate possible changes in the quality of the product before **in vivo** performance is affected. The dissolution test is also currently used as an **in vitro** bioequivalence [BE] test and for requesting biowavier of drugs. Generally dissolution profile and profile comparison establishes the similarity and/or superiority of pharmaceutical dosage forms 2. In this article **profile** comparison has been done using two model independent methods the difference factor [f1], similarity factor [f2] and dissolution efficiency [DE] 3.4.

NAP is a member of the arylacetic acid group of nonsteroidal anti-inflammatory drugs [NSAIDs] and chemically designated as 5-6-methoxy-α-methyl-2-naphthaleneacetic acid sodium. Sumatriptan succinate [SUMA] is a selective 5-hydroxytryptamine receptor subtype agonist and is chemically designated as 3-2 dimethylamino-ethyl-N-methyl-1H-indole-5-methane sulfonamide. SUMA is used in the treatment of the migraine. Naproxen sodium [NAP] is used as a prophylaxis by migraine patients. SUMA is rapidly absorbed after oral dosing its low bioavailability approximately 14% is due to pre-systemic me-
The half-life is approximately 2 h and elimination occurs mainly by non renal routes. SUMA and NAP drugs may be prescribed concomitantly and has been proven to provide higher efficacy[5 – 8]. In the present study[8] we have developed a fixed dose combinational drug containing NAP and SUMA and studied its in vitro dissolution profile.

SUMA and NAP were chosen for preparing a combinational formulation based on the preliminary work carried out by many scientist world wide[8] who have shown that a combination drug is effective for acute treatment for migraine headaches inflammation and vasodilation. The human dose of the in-house combination was fixed based on the results of LD50 studies in rodents and the literature on bioavailability of NAP and SUMA.


1 Experimental section

1.1 Chemicals

Certified reference standards of NAP[25] Batch No. 10N261006 assay potency of 98.6%[8] and SUMA[26] Batch No. SUM2006A09 assay potency of 97.4%[8] were obtained as gift samples from Divis Pharmaceuticals[27] India and Sun Pharmaceuticals[28] India respectively. Aceclofenac[29] ACE[30] Batch No. 1074869214 assay potency of 98.3%[31] was procured from Amoli Organics[32] Mumbai[33] India[34]. The pharmaceutical individual formulations were obtained commercially.


Water was purified using millipore® system. HPLC grade acetonitrile and LR grade triethylamine[52] orthophosphoric acid were purchased from Merck® Darmstadt[53] Germany[54]. Hydrochloric acid[55] HCl[56] potassium chloride[57] KCl[58] sodium hydroxide[59] monobasic potassium dihydrogen orthophosphate and glacial acetic acid were purchased from Ranbaxy[60] Gurgaon[61] India[62]. The 0.2 mol/L HCl pH 1.2[63] and monobasic potassium phosphate buffer[64] pH 6.8[65] were prepared according to the directions in USP 29. Potassium dihydrogen orthophosphate buffer[66] pH 4.5[67] was prepared according to FIP dissolution guidelines. These media were aerated prior to use by sonicating for 20 min.

1.2 Instrumentation

Dissolution test was performed using Electrolab TDT-08L multi-bath[68] n = 8[69] dissolution test system Mumbai[70] India[71] in accordance with USP general methods. Friability of the tablet was done using Friabilator[72] USP[73] Electrolab[74] Mumbai[75] India[76]. Tap density[77] ETD-1020 with USP I and USP II was used[78] Electrolab[79] Mumbai[80] India[81]. Tablet compression was done using rotary press B2 Rimek[82] Mumbai[83] India[84].

1.3 Tablet formulation

The optimized method for dosage form formulation is as follows[80] Granules of NAP and SUMA were prepared by the wet granulation method. A mixture of aerosil and magnesium stearate[85] 1:2 w/w was used as lubricant and Avicel pH-102 was used as diluent. Cross carmellose was used as a super disintegrant agent. The powders were blended and granulated with isopropyl alcohol. The wet mass was passed through a mesh of 1 mm aperture and the granules were dried at 60 °C for 1.5 h. The granules were compressed after passing through a mesh of 710 μm lubricated with mixture of aerosil and magnesium stearate 1:2 w/w.
Table compression was optimized by testing for strength, friability and hardness. Once optimized it was compressed at constant pressure using 12 mm diameter deep concave punches. The compressed tablets were enteric coated using 3.5% methacrylic acid Eudragit L100 for release in gastrointestinal tract and colored using sunset yellow as a coloring agent. The optimized formula used to develop the IR enteric coated tablet is given in Table 1.

Table 1 Optimized formula used to develop the immediate release tablet

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naproxen sodium</td>
<td>500 mg</td>
<td>active ingredient</td>
</tr>
<tr>
<td>Sumatriptan succinate</td>
<td>100 mg</td>
<td>active ingredient</td>
</tr>
<tr>
<td>Cross cromellose</td>
<td>40 mg</td>
<td>super disintegrant</td>
</tr>
<tr>
<td>PVP-K-30</td>
<td>40 mg</td>
<td>granulating agent</td>
</tr>
<tr>
<td>Avicel pH-102</td>
<td>39 mg</td>
<td>diluent</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>8 mg</td>
<td>lubricant</td>
</tr>
<tr>
<td>Aerosil</td>
<td>4 mg</td>
<td>lubricant</td>
</tr>
<tr>
<td>Eudragit L100</td>
<td>3.5%</td>
<td>enteric coating</td>
</tr>
<tr>
<td>Isopropyl alcohol</td>
<td>Q. S.</td>
<td>granulating fluid</td>
</tr>
<tr>
<td>Color</td>
<td>Sunset yellow</td>
<td>coloring agent</td>
</tr>
</tbody>
</table>

* Quantity used for formulating each tablet.

1.4 Chromatographic conditions

An HPLC method with UV detection was developed to quantify NAP and SUMA in presence of the tablet excipients. A good chromatographic separation was achieved using a Phenomenex C18 column 250 mm x 4.6 mm 5 μm. UV detection at 228 nm was set based on the highest absorbance observed with overlapping UV sample method. The column temperature was maintained at 25 °C. The mobile phase was prepared daily by dissolving 6.8 g of potassium dihydrogen orthophosphate in 1 L of milli-Q water and adjusting the pH to 5.0 with TEA. The buffer and acetonitrile were mixed in the ratio of 40: 60 v/v respectively. The mobile phase was filtered using a 0.45 μm membrane filter Sartorius Goettingen Germany and degassed with ultrasonic bath for 20 min. The injection volume was 20 μL and the run time was 12 min. The mobile phase flow rate was 0.5 mL/min.

1.5 Dissolution test conditions

NAP and SUMA sink conditions were determined in different dissolution medium. The solubility of the drug was tested using an amount of NAP and SUMA equivalent to three times of the dose in the pharmaceutical formulation in 900 mL of 0.2 mol/L HCl pH 1.2 phosphate buffer pH 4.5 and pH 6.8.

Dissolution testing was performed in USP 29 testing apparatus type 2 rotating at 50 r/min and 900 mL of the different dissolution media. The medium which was deaerated using an ultrasonic bath for 20 min was maintained at 37 ± 0.5 °C. The dissolution vessels were covered to minimize evaporation. Aliquot of 5.0 mL were withdrawn at 0.5, 1.0, 1.5, 2.0 h and the same volume of the medium was replaced at 37 ± 0.5 °C so as to maintain constant vessel volume.

The standard solution used in all dissolution tests was prepared by weighing accurately 25 mg of NAP and 5 mg of SUMA that was transferred to a 100 mL volumetric flask and diluted with dissolution medium. This was further diluted to obtain the final concentration of 5 mg/L of NAP and 1 mg/L of SUMA. The solution was filtered using 0.2 μm membrane filter before injecting into the column.

The dissolution profiles were obtained after determining the tests for the best dissolution condition. The content uniformity of the three products tested was assessed individually using twelve units of each product. The percentage found was used to calculate the percentage drug release in each time profile. The standard solution preparation for content uniformity was carried out by weighing 500 mg of NAP and 100 mg of SUMA which was transferred to separate 100 mL volumetric flasks make up with water. Pipette out 5 mL each of these solutions and transfer to a 50 mL volumetric flask and diluted with water. This solution was further diluted to obtain a final concentration of 5 mg/L and 1 mg/L respectively. Sample solutions were prepared by taking equivalent weight of one tablet and transferring into 100 mL volumetric flask containing 80 mL of water. This was kept in the ultrasonic bath for 10 min and the volume was further adjusted to 100 mL with water. Further dilutions were done to obtain a final concentration of 5 mg/L and 1 mg/L respectively. The standard and the sample solutions
were filtered using a 0.2 μm membrane filter before analysis by HPLC.

The dissolution profiles were compared by two model independent methods—the difference factor \( f_1 \), the similarity factor \( f_2 \), and dissolution efficiency \( DE \). As per FDA Guideline for “Dissolution Testing of IR Solid Oral Dog Form” the \( f_1 \) calculates the percent \( \% \) difference between the two curves at each time point and is a measurement of the relative error between the two curves

\[
 f_1 = \left( \frac{\sum_{n=1}^{t} |R_t - T_t|}{\sum_{n=1}^{t} R_t} \right) \times 100
\]

Where \( n \) is the number of time points, \( R \) is the dissolution value of the reference batch at time \( t \), and \( T \) is the dissolution value of the test batch at time \( t \).

The \( f_2 \) is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in the percent \( \% \) dissolution between the two curves.

\[
 f_2 = 50 \times \log \left( \left[ 1 + \frac{1}{n} \sum_{n=1}^{t} R_t - T_t \right]^{-0.5} \right) \times 100
\]

The \( f_1 \) is zero when the test and drug reference profiles are identical and increase proportionally with the dissimilarity between the two dissolution profiles. The \( f_2 \) is 100 when the test and reference profiles are identical and tends to 0 as the dissimilarity increases. Two dissolution profiles are declared similar if the \( f_1 \) is between 0 and 15 and if the \( f_2 \) is between 50 and 100\% \[3\] [4]. The DE was calculated from the area under the dissolution curve at time \( t_i \) measured using the trapezoidal rule and expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time.

The comparison of the dissolution profiles for different products cited in section 1.1 was realized. The results of the DE, the \( f_1 \) and the \( f_2 \) are presented. Since the Product A is in-house formulated the \( f_1 \) and the \( f_2 \) were calculated between Product A and B for NAP A and C for SUMA. The results of the \( f_1 \) and the \( f_2 \) for the comparison of product A-B and product A-C showed that the profiles are similar. The dissolution efficiency was calculated for all products.

1.6 Validation

In order to demonstrate the method was adequate for dissolution test purposes it was validated thoroughly for stability, specificity, linearity, precision, accuracy and robustness parameters \[2\] [2].

1.6.1 Stability

The stability of standard and sample solutions which were prepared using 0.2 mol/L HCl pH 1.2 and phosphate buffer pH 6.8 was evaluated after storing the solutions for 24 h at 4 °C and at room temperature. The chromatograms were obtained by the HPLC method.

1.6.2 Specificity

It was evaluated by preparing a placebo from the reference individual standards in-house combinational formulation. The placebo was transferred to vessel containing 900 mL of dissolution media stirred at 37 ± 0.5 °C for 1 h at 50 r/min using paddle. Samples of this solution were filtered first with quantitative filter and then with a 0.2 μm membranes filter and analyzed by HPLC. The chromatogram was checked for placebo interference at the retention time of the drugs and IS.

1.6.3 Linearity

Aliquots of 1, 3, 5, 7, 9 and 10 mg/L solution of NAP and SUMA reference standard were prepared with phosphate buffer pH 6.8. Each linearity point concentration was prepared separately using the reference standards. This was prepared in triplicate. The linearity was evaluated by linear regression analysis which was calculated by the least square regression method and analysis of variance ANOVA.

1.6.4 Precision

Repeatability and intra-day precision were used to assess the precision of the method. Repeatability was evaluated through relative standard deviation RSD obtained by triplicate injection of NAP SUMA and compared with the recovery obtained for two different days and the intra-day precision using different HPLC equipments.

1.6.5 Accuracy

The accuracy study was performed in triplicate by adding 500 mg of NAP and 100 mg of SUMA reference standard to placebo sample. The disso-
lution test was done for 30 min using 900 mL of dissolution medium of phosphate buffer pH 6.8 type 2 apparatus rotating at 50 r/min. Aliquots of 5.0 mL were pipette diluted accordingly and filtered with 0.2 μm membrane filter and analyzed by HPLC.

1.6.6 Robustness

It was evaluated by making small but deliberate changes to the method parameters. An experimental design was used to determine how the influence of dissolution medium affects the profile release of NAP and SUMA from the tablets.

2 Results and discussion

The discriminatory power of the dissolution method depends on the ability of method to detect changes in the drug product. Drug solubility and solution stability are important properties to be considered when selecting the dissolution medium [2]. The sink conditions tested showed that NAP and SUMA combination bulk was soluble in 0.2 mol/L HCl pH 1.2 phosphate buffer with pH 4.5 and pH 6.8. Dissolution tests for the in-house combinational formulation individual commercial formulation of NAP and SUMA tablets were then performed using this dissolution medium at the stirring speed of 50 r/min to investigate the drug release in each medium.

The initial parameters for solution stability must be established prior to the completion of any dissolution samples. The multimedia was selected based on the physiological pH range and chromatograms for each medium were obtained. The solutions remained stable in all dissolution media tested for the time period specified and no degradation products were observed in any chromatogram. It was therefore possible to guarantee the integrity of the drug during the analysis time.

The specificity analysis revealed that the HPLC method did not suffer interference by the formulation excipients since there was no interfering peak in the retention time of SUMA 4.85 ± 0.02 min ACE 5.74 ± 0.01 min and NAP 7.93 ± 0.02 min Fig. 1. The chromatographic peak purity was applied for NAP 99.998 5 and SUMA 99.951 2 peaks which demonstrated that there were no impurity peaks.

![Fig. 1 Chromatogram of the drug sample](image1)

1. sumatriptan succinate SUMA 2. aceclofenac ACE IS 3. naproxen sodium NAP.

The dissolution test conditions were selected based on a screening study with USP II apparatus. The tablets were tested in 900 mL of 0.2 mol/L HCl pH 1.2 4.5 and 6.8 phosphate buffer with pH 4.5 and pH 6.8 Fig. 2. As expected for en-

![Fig. 2 Dissolution profiles of NAP and SUMA with Product B Product C using phosphate buffers with pH 1.2 4.5 and 6.8 USP II apparatus rotating at 50 r/min](image2)
teric coated IR tablets\[ the dissolutions of NAP and SUMA were rapid and essentially complete within 2 h at pH 6.8\] expected to be present in gastrointestinal tract. However\[ at pH 1.2 there was no release and at pH 4.5 there was slow release indicating that the drug is completely released only in gastrointestinal tract. Hence\[ USP II apparatus with paddle rotating at 50 r/min was selected as the dissolution apparatus and 900 mL of buffer\[ pH 6.8\] was chosen as the dissolution medium.

To assess the linearity\[ three standard curves for NAP and SUMA were constructed\[ individually\] plotting concentrations\[ mg/L\] versus absolute areas\[ the correlation coefficients of 0.992 9 and 0.993 1\] were obtained respectively. The slopes obtained were 1.179 $\times 10^{-1}$ and 1.279 $\times 10^{-1}$\] and the intercepts were 8.942 $\times 10^{-3}$ and 9.358 $\times 10^{-3}$ respectively. ANOVA showed significant linear regression and no-significative linearity deviation\[ $P < 0.05$\]. These data indicate that the method is linear for NAP and SUMA\[ Table 2\].

The precision of the dissolution tests\[ Table 2\] were evaluated through repeatability and intra-day precision. The repeatability demonstrated RSDs of $0.998 \pm 0.194\%$ and $0.607 \pm 0.249\%$ intra-day precisions of $1.060 \pm 0.264\%$ and $1.426 \pm 0.254\%$ inter-day precisions of $0.659 \pm 0.268\%$ and $0.085 \pm 0.568\%$ for NAP and SUMA respectively. These results demonstrated good precision of the method for the dissolution test.

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<tbody>
<tr>
<td>NAP</td>
<td>Slope: 0.1179, Intercept: 8.942 $\times 10^{-1}$, Residual error: 0.0042, $r^2$: 0.9929</td>
<td>Precision: RSD: $0.998 \pm 0.194%$, Repeatability: 1.060 $\pm 0.264%$, Intra-day: 0.659 $\pm 0.268%$, Inter-day: 0.3 $\pm 1.00$</td>
<td>0.3</td>
<td>1.00</td>
</tr>
<tr>
<td>SUMA</td>
<td>Slope: 0.1279, Intercept: 9.358 $\times 10^{-1}$, Residual error: 0.0044, $r^2$: 0.9931</td>
<td>Precision: RSD: $0.607 \pm 0.249%$, Repeatability: 1.426 $\pm 0.254%$, Intra-day: 0.085 $\pm 0.568%$, Inter-day: 0.6 $\pm 1.60$</td>
<td>0.6</td>
<td>1.60</td>
</tr>
</tbody>
</table>


The accuracy\[ Table 3\] expressed good agreement between the accepted value and the value found. The recoveries were $96.99 \pm 3.483\%$ and $95.87 \pm 2.290\%$ for NAP and SUMA respectively. The accuracy of the method was considered acceptable based on its intended use.

In the evaluation of the robustness of the method\[ the presence of possible air bubbles in the dissolution medium had no interference in the dissolution profile of the NAP and SUMA tablets. These results demonstrate that the method is robust.

The mean values found for the uniformity of content to Product A\[ were 96.125\% and 98.685\% RSD = 1.40\% and 1.45\% for NAP and SUMA\[ respectively\] Product B was 101.24\% RSD = 2.36\% and Product C tablets 104.71\% RSD = 1.11\%.

### 3 Conclusion

The dissolution test developed and validated for NAP and SUMA combination tablets was considered satisfactory. The drugs were found to be stable during analysis. The dissolution conditions were 900 mL of phosphate buffer\[ pH 6.8\] medium at 37 °C using USP II apparatus\[ stirring speed of 50 r/min and filtration with 0.2 μm membrane filters. The comparison of the obtained dissolution profiles was realized by DE and the factors $f_1$ and $f_2$ which showed that the profiles were similar for tablet products A-B and A-C\[ Table 4\]. This method demonstrated to be adequate for in vitro studies of NAP and SUMA combination dosage form\[ since there is no official monograph\] collaborating to the official codes.
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<tr>
<td>DE</td>
<td>72.93</td>
<td>53.41</td>
<td>73.08</td>
</tr>
<tr>
<td>f₁</td>
<td>0.43</td>
<td>4.89</td>
<td>- -</td>
</tr>
<tr>
<td>f₂</td>
<td>91.28</td>
<td>67.07</td>
<td>- -</td>
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References