ORIGINAL ARTICLE

# Study of Drug Release Profiles Obtained by Auto-dilution and Auto-mixing of Dissolution Aliquots of Naproxen Sodium Tablets IP: A Statistical Evaluation

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## Abstract

**Aim:** To study the drug release profiles obtained by auto-dilution and auto-mixing of dissolution aliquots of naproxen sodium tablets IP and evaluate it statistically. **Materials and Methods:** Naproxen sodium active pharmaceutical ingredient was procured from Divis Laboratory (India). The drug dissolution media was prepared by diluting media concentrates (Jawa buffer concentrate pH 7.4, Electropharma) to obtain phosphate buffer pH 7.4. Investigations are necessary to understand the effect of dilution and mixing efficiency on the ultra-violet (UV) analysis of in-vitro dissolution aliquots of high dose drugs. In the present research, two independent sets of studies were carried out for the determination of the percentage of drug released from high-dose naproxen sodium tablets. **Results and Discussion:** Dissolution studies and subsequent drug analysis for high-dose oral solid dosage form is a challenge to a drug analyst. Automation in dilution and mixing of collected sample can offer a measure toward streamlining the dilution and mixing procedure resulting in uniformity of drug dissolution profile. Experimental data from manual as well as automated methods of sampling, dilution and mixing were obtained and evaluated statistically for variations in data point values. The similarity and difference factors were found to be 93.05 ($f_2$) and 1.10 ($f_1$), respectively. Both the methods of dissolution aliquot mixing were compared by applying the two-tailed paired Student's *t*-test. Student's "*t*" statistics showed that there was non-significant difference between the two methods ($P \geq 0.05$). **Conclusion:** The analysis of the data ensured a statistical closeness of the results obtained and empowered use of an automated dissolution system capable of diluting and effectively mixing dissolution aliquots before UV spectrophotometric analysis.

**Key words:** Automated mixing, manual mixing, naproxen sodium, spectrophotometry, student's *t*-test

## INTRODUCTION

Dissolution testing is a potential indicator of physiological variability that depends on the drug in a dissolved state in biological fluids.[1] Furthermore, dissolution test provides crucial information on physicochemical stability of the product when the dosage form is tested over the period for stability.[2] As per the quality-by-design principle, it is vital to collect information about the dosage form and its dissolution as soon as possible in the process of development. Drug release studies, require constant monitoring and careful physicochemical evaluation to obtain pharmaceutically relevant data. It is more austerely applicable to extended release formulations. Drug release monitoring for extended release formulations is an arduous task; hence,

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automation of dissolution sample collection, replenishment, dilution, and mixing of aliquots for drug analysis becomes highly desirable.

Automated methods are generally accepted as resource management tool and are successfully being employed in drug development process with accuracy. This has not just reduced the human effort in labor oriented dissolution testing but helped to increase reproducibility for the scientific evaluation of a greater number of dosage forms.[3,4] Advancements in dissolution rate testers include sample withdrawal at preprogrammed intervals with or without replenishment along with dilution and mixing. Moreover automated sampling systems may also be equipped with online auto analyzer such as ultra-violet (UV) spectrophotometer.[5,6]

As per Beer-Lambert law, an accurate UV spectrophotometric analysis of dissolution aliquots requires UV absorbance in the range of 0.2-1.0 to achieve linear correlation coefficient close to 0.999 (Beer Lambert's law).[7] Hence, when using an online auto analyzer, one of the major aspects to be accounted is the dilution factor required to obtain drug concentration in Beer-Lambert range. Every drug has its unique property in terms of wavelength maxima ($\lambda_{max}$), and its corresponding absorbance value at selected $\lambda_{max}$. Moreover, dilution of the dissolution samples of high dose drugs has been widely reported in literature for the purpose of accurate analysis. Therefore, automation of dissolution sample dilution along with efficient mixing before drug analysis is highly recommended.[8]

In the present study, independent sets of studies were carried out for percentage drug release analysis of naproxen sodium tablets employing manual and automated aliquot dilution followed by the mixing. The US Food and Drug Administration, USP and GLP guidelines require validation and comparative assurance between the manual and the automated results. Hence, after carrying out the dissolution study and UV spectrophotometric analysis, the results were used to calculate difference factor ($f_1$) and similarity factor ($f_2$). Subsequently, the data was subjected to two-tailed paired *t*-test, as an additional tool for evaluation.[9,10]

## EXPERIMENTAL

### Materials and instruments

Naproxen sodium active pharmaceutical ingredient was procured from Divis Laboratory (India). PVDF filters (0.45 µm × 33 mm) were received as gift sample from Merck, Millipore (India). All chemicals and buffers used were of analytical grade. The dissolution media was prepared by diluting media concentrates (JAWA pH 7.4, Electropharma) to obtain phosphate buffer pH 7.4. Manual dissolution testing was carried out using Electrolab 08Lx USP Apparatus 2, whereas Automated sampling, dilution and mixing were carried out using Electrolab 08Lx USP Apparatus 2 equipped with offline sampling system comprising a syringe pump (ESP-84) and sample collector (ESC-08s). UV spectrophotometer used was PerkinElmer Lambda 25 for spectrophotometric measurements, PerkinElmer (USA). Electrolab Degasser (EMP-21) was used to degas the dissolution media.

### Ultra-violet analytical method development

The standard solution of naproxen sodium was prepared by dissolving accurately weighed (25 mg) naproxen sodium in 100 ml of phosphate buffer pH 7.4.[9] From above stock, different aliquots were taken separately and diluted to obtain a concentration in the range of 20-160 µg/ml.

UV spectrophotometric detection was carried out at $\lambda_{max}$ of 330 nm. The developed UV method was validated for specificity, linearity, range, accuracy, and precision.

#### *Stability of standard and sample solutions*

The stability of standard stock solution and tablet solutions in dissolution media was evaluated at room temperature (25°C ± 1°C) and refrigerated temperature (8°C ± 2°C) up to 72 h.

#### *Specificity*

Specificity is the ability of an analytical method to accurately measure the response of the analyzed compound without any interference from the sample matrix. An amount of naproxen sodium equivalent to the content of one tablet was transferred to a vessel containing 900 ml of medium and stirred for 1 h at 100 rpm using USP Apparatus 2. Similarly, naproxen sodium tablets were also studied in 900 ml of medium and stirred for 1 h at 100 rpm using USP Apparatus 2. Collected samples were filtered and absorbance was measured.

#### *Linearity*

Different aliquots of naproxen sodium standard solution (1000 µg/ml) were transferred to 10 ml volumetric flasks and diluted with phosphate buffer pH 7.4 up to the mark to achieve five different concentrations: 20, 40, 80, 120, and 160 µg/ml.

The solutions were analyzed in triplicate for 3 consecutive days. Linearity was determined by linear regression analysis using analysis of variance.

#### *Accuracy and precision*

Accuracy was evaluated by the recovery of known amount of naproxen sodium reference substance added to the known concentrations of drug present in the tablet matrix. Aliquots of 1 ml, 4 ml, and 8 ml of the standard solution (1 mg/ml) and a known amount of crushed tablet equivalent to one drug dose were added to the vessels (900 ml) containing dissolution medium; this mixture was agitated for 60 min with paddle at 100 rpm. The analyses were carried out in

duplicate on 3 different days. Repeatability (intraday) and intermediate precision (interday) were evaluated based on the relative standard deviation (RSD) from the recovery data.

### *In-vitro* dissolution testing

Dissolution testing was performed using USP Apparatus Type 2 with paddles at 100 rpm, in 900 ml of phosphate buffer pH 7.4 at 37°C ± 0.5°C as reported in USNF. Manual sampling aliquots (10 ml) were withdrawn at 10, 20, 30, 40, 50, and 60 min and were immediately filtered. Samples were diluted and subjected to two different mixing conditions. Manual mixing was carried out in volumetric flasks. For the automated system, samples were withdrawn by the autosampler attached to syringe pump, diluted and mixing was carried out by high-speed rotation of dilution needle for 30 s. These diluted and mixed samples were analyzed for drug concentration.

## RESULTS AND DISCUSSION

The objective of the present investigation was to establish a statistically significant correlation between role of manual mixing and automatized mixing with respect to the assessment of percent drug released from the formulation. Dissolution samples with 2 different mixing treatments were analyzed by reported UV spectrophotometric method. To assess the % drug released from the formulations; UV analytical method was developed at 330 nm.

### Dissolution testing conditions

*In vitro* dissolution study for naproxen sodium tablets is reported in USP. Naproxen sodium has good aqueous solubility, that is, approximately 15.9 mg/ml. The solubility of 550 mg of naproxen sodium was assessed in the phosphate buffer pH 7.4. It was freely soluble in the dissolution media, and this ensured that sink conditions will be maintained for the specified dose of naproxen sodium in 900 ml of phosphate buffer pH 7.4. Hence, dissolution study was performed in this media to achieve experimental results.

Naproxen sodium samples were evaluated for stability in dissolution media. It was observed that drug samples showed no significant change in UV absorbance in pH 7.4 phosphate buffer after 2 h at room temperature as well as for 72 h under refrigeration. The values ranged from 99.1% ± 0.20% to 100.01% ± 0.20% for solutions at room temperature, and from 100.0% ± 0.24% to 100.5% ± 0.22% under refrigeration. This ensured solution stability of drug during the period of dissolution testing. UV spectrophotometric scan showed no degradation and impurity peak.

The specificity of the dissolution test method using a UV detector demonstrated no excipient interference. UV spectrophotometric analytical method was found to be linear at the concentration range of 20–160 µg/ml. Correlation coefficients ($r^2$) was found to be 0.999. The equations for the calibration curve was $y = 0.005\ x + 0.036$. The measured accuracy was considered adequate in the range of 96.2-104.1% for naproxen sodium. Repeatability and intermediate precision were evaluated over 3 days. The low RSD values (≤2%) demonstrated the good precision of the method.

### Analysis of percent drug dissolved and statistical evaluation

A dissolution study was conducted as per the parameters specified earlier. 2 ml of dissolution aliquots were collected at 10, 20, 30, 40, 50, and 60 min, diluted up to 20ml with phosphate buffer pH 7.4 and analyzed. In case of manual system, aliquots were thoroughly mixed with diluents by hand shaking whereas in automated system sample dilution needle mixes the sample by high speed rotational movement for preprogrammed duration. After mixing the aliquots meticulously, they were subjected to UV spectrophotometric analysis. Figures 1 and 2 show the release profiles of manual and automated dissolution studies, respectively. The results and observations of release study showed closeness in data point values and to obtain any further statistical significance

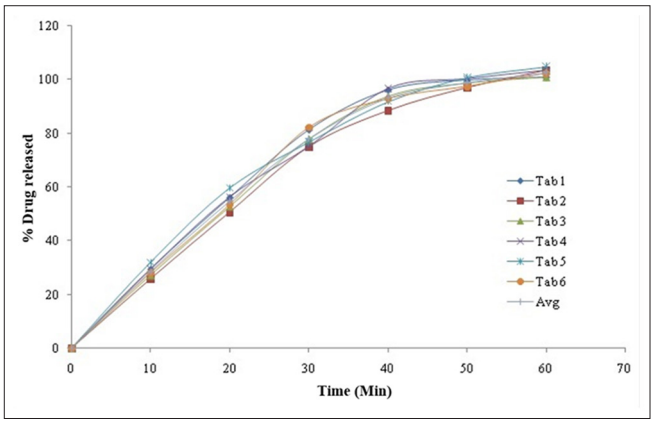


**Figure 1:** Percentage drug release obtained on manual mixing of dissolution aliquots with the diluents

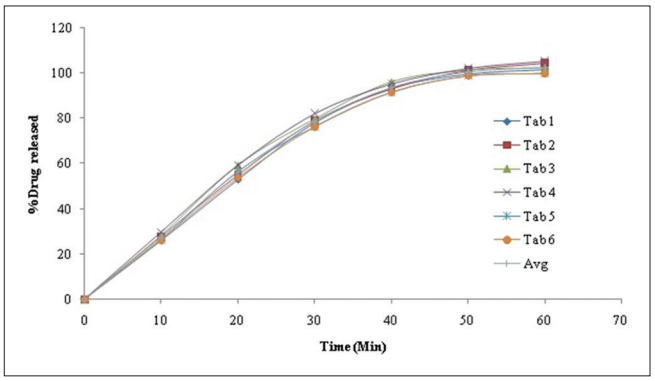


**Figure 2:** Percentage drug release obtained on automated mixing of dissolution aliquots with the diluents

the release profiles were subjected to calculation of similarity factor and difference factor along with Student's *t*-test analysis.

The drug-release profiles obtained by manual and automated mixing were compared using the difference factor ($f_1$ = 1.10) and similarity factor ($f_2$ = 93.05).

In the current study, model-independent approach has been utilized to compare the data. It is recommended for use in most guidance documents published by regulatory agencies for the comparison of dissolution profiles. The difference factor ($f_1$) is calculated using Equation 1 and the similarity factor ($f_2$) calculated using Equation 2. The similarity factor fits a result between 0 and 100, and the value for $f_2$ is 100 when the test and reference profiles are identical.[11-13] The fit factors are advantageous to use for dissolution curve comparison, as they are easy to compute and provide a single number for the purposes of describing two dissolution profiles.

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

$$f_2 = 50\log\left\{\left[\left(1+\left(\frac{1}{n}\right)\sum_{t=1}^{n}(R_t - T_t)^2\right)\right]^{-0.05} \times 100\right\} \quad (2)$$

Where,

$n$ = the sample number,

$R_t$ = the percent drug dissolved from the reference product at t time points.

$T_t$ = the percent drug dissolved from the test product at t time points.

The results indicate that the curves are similar because $f_1$ was less than 15 and $f_2$ was >50.

The two-tailed paired Student's *t*-test was applied to compare both the set of data. The probability value *$P < 0.05$, was considered statistically significant value for the study groups. Non-significant difference was found with the "*t*" statistics since $P = 0.2349$ is higher than the critical statistical value ($P = 0.05$). Therefore, Student's *t*-test confirms that the two dissolution profiles have no significant difference. This ensured that manual and automated system results in similar dissolution data.[14]

Results of these practical and statistical studies have created a confidence in the use of a fully automated dissolution type II apparatus with the unique feature of dilution and mixing of the dissolution aliquots [Table 1]. The system utilized auto-dilution and mixing with a rotating needle for uniform sample preparation. The statistical closeness of the data ensured that it's a successful approach toward complete automation of drug release studies. Based on the observations and results, we could appreciate the significance and role of sufficient, uniform mixing on the percent drug release.

In conclusion, automated methods are accepted as a resource management tool. The data collected was reliable and comparable to a manual dissolution testing and analysis.

### Determination of release kinetics

The results of *in vitro* drug release studies for manual as well as automated dissolution apparatus were fitted in various mathematical models such as zero order, first order, Higuchi's square root, Hixson-Crowell cube root law, and Korsmeyer-Peppas equation. The kinetics and mechanism of drug release from the tablets were evaluated based on the release kinetic model that best fits the release data. The data shown in Table 2 clearly indicated Hixson-Crowell model with $r^2$ value of 0.992 and 0.99 for manual and automated systems respectively. The Hixson-Crowell plot ($r^2$ = 0.99) showed a change in surface area and diameter of the tablets with the progressive dissolution of the tablet as a function of time.[15] The corresponding plot (log cumulative percent drug release vs. Log time) for the Korsmeyer-Peppas equation ensured a good linearity ($r^2$ = 0.963). The release exponent n

**Table 1:** Percentages statistical evaluation of average dissolution profiles

| Time (min) | Dissolution profile on manual system | Dissolution profile on automated system | Statistical results |
|---|---|---|---|
| 0 | 0 | 0 | Difference factor, ($f_1$=1.10) and Similarity factor, ($f_2$=93.05) |
| 10 | 28.76±2.6 | 27.50±2.58 | |
| 20 | 54.92±3.13 | 56.22±3.68 | |
| 30 | 78.14±3.85 | 78.66±4.08 | |
| 40 | 93.35±4.23 | 93.60±4.06 | "*t*" statistic value (0.2349) is higher than the table value (0.05) |
| 50 | 99.08±4.55 | 100.41±4.19 | |
| 60 | 102.74±5.26 | 102.36±4.52 | |

Data are expressed as mean±SD (*n*=6). SD: Standard deviation

**Table 2:** Release kinetics of dissolution profiles obtained from Manual and Automated system

| System | Release kinetics | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Zero order | | First order | | Higuchi plot | | Korsmeyer-Peppas model | | | Hixson-Crowell | |
| | $r^2$ | $k_o$ ($h^{-1}$) | $r^2$ | $k_1$ (h–1) | $r^2$ | $k_H$ ($h^{-1/2}$) | $r^2$ | $n$ | $k_{KP}$ ($h^{-n}$) | $r^2$ | $k_{HC}$ ($h^{-1/3}$) |
| Manual | 0.904 | 1.478 | 0.925 | 0.106 | 0.961 | 16.77 | 0.963 | 0.729 | 5.8479 | 0.992 | 0.08 |
| Automated | 0.894 | 1.49 | 0.962 | 0.078 | 0.955 | 16.96 | 0.963 | 0.729 | 5.8479 | 0.99 | 0.075 |

was 0.72, which is 0.45< $n$ <0.89 indicating anomalous (non-Fickian) diffusion from the dosage form.

## CONCLUSION

An automated dissolution method for analysis of naproxen sodium tablets was developed. The UV spectrophotometric method was used to analyze the percentage of drug dissolved versus time and presented acceptable specificity, linearity, accuracy, and precision. The estimation of percent drug release can be made more accurate and reliable by a competent automated system which is statistically comparable to the manual system. Hence, evaluation of the automated data presented above indicated that implementation of automated systems in quality control groups can make the dissolution process much faster, simpler, accurate, and comparable to a manual system requiring human effort.

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