

# Development of a Dissolution Method for Modified Release Tablets Containing an Insoluble Active Substance

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**Objective:** The aim of the present work is to develop a discriminative dissolution method for a practically insoluble pharmaceutical active substance such as indapamide.

**Methods:** Dissolution testing was performed in compliance with USP, using USP apparatus 2. The proper dissolution medium and the optimal rotation per minutes of the apparatus were optimized. In order to quantify the dissolution of indapamide from modified release tablets, a high liquid chromatographic method was developed and validated.

**Results:** An HPLC method was developed in order to provide adequate specificity trying several column types and different mobile phases. We selected the proper dissolution medium based on indapamide solubility, we determined the optimal speed of rotation of the dissolution tester for the indapamide in the selected medium, then we proved the discriminating power of the developed dissolution method.

**Conclusions:** A robust and discriminating HPLC method for analyzing dissolution samples containing indapamide was developed and successfully validated.

**Keywords:** indapamide, dissolution profile, discriminative dissolution method, similarity fit factor  $f_2$

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## Introduction

In drug design it is very important to establish the solubility of the active pharmaceutical ingredient, and to anticipate its *in vivo* solubility from the dosage form with reliable *in vitro* methods, taking into account the chemical structure and physical-chemical properties. The most challenging types of drugs are the poorly soluble pharmaceutical substances, their formulation being focused on obtaining an enhanced bioavailability [1,2].

Indapamide is an antihypertensive/diuretic drug. It has an antihypertensive action causing a drop in systolic, diastolic and mean blood pressure. It has an extrarenal antihypertensive action with a decrease in vascular hyperreactivity and a reduction in total peripheral and arteriolar resistance. There is also a direct renal diuretic action. Indapamide is the first in its antihypertensive/diuretics class, the indolines [3]. Indapamide is practically insoluble in water [4], and it is rapidly and almost completely absorbed after oral administration [5]. Based on these properties indapamide belong to class II in the Biopharmaceutics Classification System (BCS). The BCS is a scientific framework for classifying a drug substance based on its aqueous solubility and intestinal permeability [6].

A discriminative dissolution method is a very good tool in drug design to define the appropriate qualitative and quantitative composition of the final medicinal product [7]. Our goal was to develop a discriminative dissolution method for a modified release tablet containing 1.5 mg indapamide.

## Material and method

Indapamide (Bioindustria, Italy) was of high purity standard. The test product was developed in the laboratories of the Development Department of Gedeon Richter Romania S.A. The other chemical reagents were also analytical grade purity: potassium dihydrogen phosphate (Merck, Germany), disodium hydrogen phosphate dihydrate (Merck, Germany), sodium hydroxide (Merck, Germany), 1 octanesulfonic acid sodium salt monohydrate (Merck, Germany), glacial acetic acid (VWR BDH Prolabo), acetonitrile (VWR BDH Prolabo), sodium acetate (Merck, Germany).

## In vitro dissolution study

Dissolution testing was performed in compliance with USP [8]. The dissolution test was performed with Erweka DT 800 LH multi-bath (n=6) dissolution system with auto sampler (Heusanstamm, Germany), in brown dissolution test vessels to avoid indapamide photo degradation [4].

The final method conditions were: apparatus 2, 900 ml dissolution medium, at a paddle speed of 100/75 rotations per minute, consecutive dissolution mediums of 0.1 M hydrochloric acid, and phosphate buffer pH=6.8 (potassium dihydrogen phosphate 0.2 mol/l and disodium hydrogen phosphate dihydrate 0.2 mol/l mixed in 510:490 volume ratio, then the pH was adjusted to 6.8±0.5% with sodium hydroxide 50% solution) maintained at 37±0.5 °C. A sample volume of 1.5 ml was taken out with auto sampler through 10 µm Poroplast and 0.45 µm PTFE filter in brown HPLC vials. The samples were collected at time points of 2, 4, 8, 12, 16 hours.

In order to appreciate the discriminatory power of the proposed dissolution method, several tablet batches manufactured with different type of hydroxypropyl methylcellulose with the same viscosity (3000–5600 Cps) were tested. Four types of 4000 CP hydroxypropyl methylcellulose were tested: HPMC-Methocel K4M Premium (Colorcon, UK), HPMC-Methocel K4M CR Premium (Colorcon, UK), Metolose 60SH-4000 (Shin-Etsu, Japan), Metolose 90 SH 4000 (Shin-Etsu, Japan). The dissolution profile data were mathematically compared using the similarity fit factor  $f_2$ .

### HPLC analysis

Analysis of dissolution samples was performed on an Elite LaChrom Merck Hitachi HPLC system equipped with a L2130 quaternary pump, L2200 auto sampler, L2455 DAD detector. Analytical separation was performed on Hypersil ODS2, 150×4.6 mm, 5  $\mu$ m particle size. The mobile phase was a 1.08 g l octanesulfonic acid sodium salt monohydrate dissolved in 700 ml water, 10 ml glacial acetic acid 100% and 300 ml acetonitrile, and was pumped at a flow rate of 1.5 ml/min. The injection volume was 50  $\mu$ l, the run time 10 minutes. Detection was achieved at 242 nm. Data integration was performed with EzChrom Elite 3.2.1 software.

The method was validated by evaluating the following parameters: selectivity, linearity, accuracy, precision, solution stability, robustness.

**Selectivity:** the chromatograms of the placebo, dissolution medium I, dissolution medium II, and the mobile phase. The method is considered selective for determination of active substance content if, under normal conditions, the placebo, dissolution medium I, dissolution medium II, and the mobile phase have no detectable signal at the retention time of the active substance.

**Linearity:** we verified the linear relationship between known concentrations of the reference substance and the peak areas in the range of 5–120% active substance for dissolution medium I, and 10–120% active substance for dissolution medium II in 6 measuring points, for two independent dissolution tests for each dissolution medium.

**Accuracy:** known amounts of indapamide (reference substance) were added to the placebo for 6 measuring points within the linearity range. The results were evaluated with regression analysis and recovery% was calculated. The method has to be accurate in the linearity range.

**Precision:** the analysis was repeated in 2 days, by 2 analysts with 2 replicate injections for each sample.

**Solution stability:** the percentage of found concentration after 16 hours was measured.

**Robustness:** the effect of changing the column temperature and the mobile phase composition was verified.

## Results

### HPLC analysis – optimization of the chromatographic conditions for sample and method validation

An HPLC method was selected for dissolution sample anal-

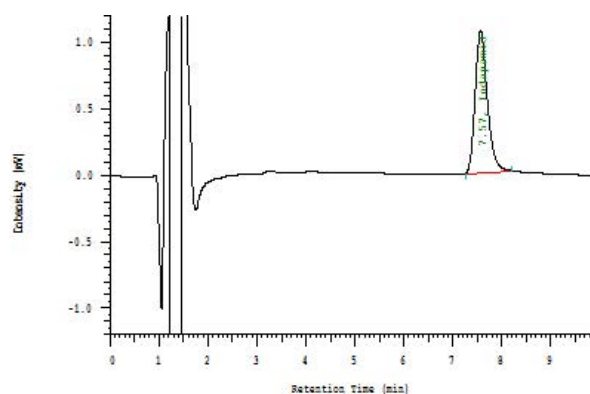


Fig. 1. Chromatogram of indapamide in dissolution medium

ysis for a better accuracy of the results. The HPLC method was developed in order to provide adequate specificity and short run time, trying several column types and different mobile phases. A typical chromatogram of indapamide sample in optimized conditions is shown in Figure 1.

The HPLC method validation included: specificity, linearity, accuracy, precision, solution stability, robustness. No interferences from excipients or dissolution medium with the indapamide peak were observed through the analysis of placebo formulation, confirming the selectivity of the method. The calibration curve showed a good correlation in the concentration range of 0.07–2.00  $\mu$ g/ml in the first dissolution medium, and 0.15–2.00  $\mu$ g/ml in the second dissolution medium. The statistical data obtained during validation were the following:

**Linearity:** equation of the calibration curve in dissolution medium I:  $y=91236.02x-545.2945$ , correlation coefficient: 0.997. Equation of the calibration curve in dissolution medium II:  $y=101023.6x-493.9913$ , correlation coefficient: 0.9996.

**Accuracy:** equation of the calibration curve in dissolution medium I:  $y=1.010147x-0.008972$ , correlation coefficient: 0.9996. Equation of the calibration curve in dissolution medium II:  $y=1014878.6x-0.00433$ , correlation coefficient: 0.9993.

**Precision:**  $F_{\text{calc}} < F_{\text{crit}}$ .

**Robustness:** the system suitability test by changing column temperature was in the acceptance limits. On the basis of the system suitability data the method was not robust for little changes in the mobile phase composition, but it was robust for increased acetonitrile ratio in the mobile phase composition.

### Development of dissolution methodology

Selection of dissolution medium to achieve adequate solubility and stability of indapamide was critical for this dissolution method.

Based on European Pharmacopoeia guidance on dissolution testing, the following dissolution specification for indapamide has been proposed: at 4 hours, 17–27%; at 8 hours, 45–65%; at 16 hours, min 75% [4]. The following dissolution media were screened:

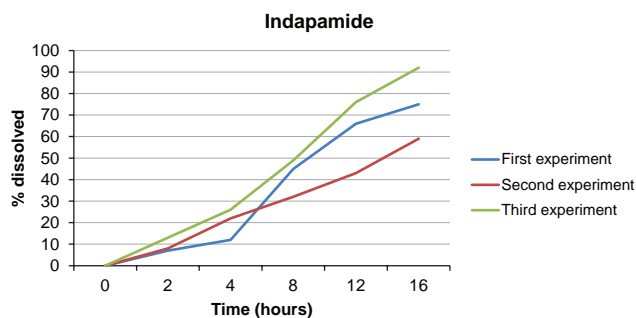


Fig. 2. Comparison of dissolution profiles of indapamide modified release tablets using different dissolution media

1. Phosphate buffer pH=6.8, 900 ml, 75 rpm (first experiment);
2. Acetate buffer pH=4.5, 900 ml (second experiment);
3. 0.1 M hydrochloric acid, 900 ml, phosphate buffer 6.8, 900 ml (third experiment).

Note that the dissolution rate of indapamide is dependent on the pH of the dissolution media (Figure 2). The results show that 0.1 M hydrochloric acid, 900 ml, followed by phosphate buffer 6.8, 900 ml, dissolution media was suitable for our developed product, only in this case the obtained results being in the proposed specification limits.

After we have chosen the optimal dissolution medium, we have determined the optimal speed of rotation of the dissolution tester for indapamide in the selected medium. The following rotation speeds were tried:

1. 100 rpm in the first medium/50 rpm in the second medium;
2. 100 rpm in the first medium/60 rpm in the second medium;
3. 100 rpm in the first medium/75 rpm in the second medium;
4. 100 rpm in the first medium/100 rpm in the second medium.

Dissolution profiles obtained on 100/75 rpm and 100/50 rpm has proper in vitro dissolution kinetics for modified release tablets (Figure 3), but the results obtained with 100/50 rpm were not within the proposed specification limits, so 100 rpm in the first medium, and 75 rpm

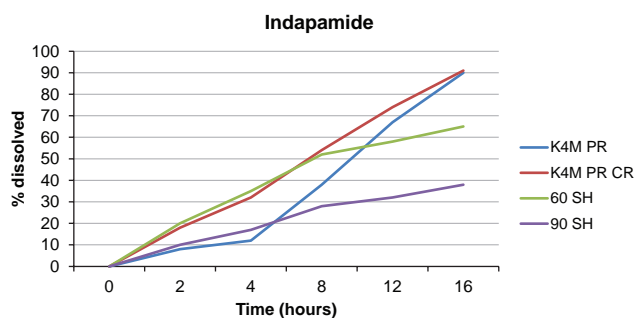


Fig. 4. Comparison of dissolution profiles of indapamide modified release tablets formulated with different type of hydroxypropyl methylcellulose

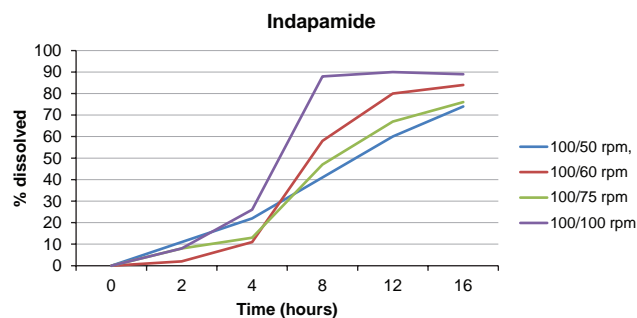


Fig. 3. Comparison of the dissolution profiles of indapamide modified release tablets using different rotation speed

in the second medium were selected as optimal speed rotations.

The obtained dissolution profiles showed differences between batches prepared with different types of hydroxypropyl methylcellulose with viscosities within the same range (Figure 4). The dissolution profile data were also mathematically compared using the similarity fit factor  $f_2$ . This fit factor directly compares the difference between percentage of dissolved drug per unit time for a test and a reference product, respectively. The fit factor,  $f_2$ , is defined by the following:

$$\text{Similarity factor } f_2 = 50 \times \log \left[ \left( 1 + \frac{1}{n} \sum_{t=1}^n (R_t - T_t)^2 \right)^{-0.5} \times 100 \right]$$

where  $R_t$  and  $T_t$  are the average values of the two data sets at time point  $t$  and  $n$  is the total number of time points used for calculation. The concept of the  $f_2$  approach was described by Moore and Flanner [9]. Two profiles are considered dissimilar when  $f_2$  is less than 50. The similarity increases if the  $f_2$  value increases above 50 and approaches 100. We have obtained the following results: 47 between batches prepared with K4M PR-CR and with K4M PR; 45 between batches prepared with K4M PR-CR and 40SH, and 28 between batches prepared with 90 SH and K4M PR-CR. These results confirm that the dissolution test procedure has discriminating power for the different type of HPMC.

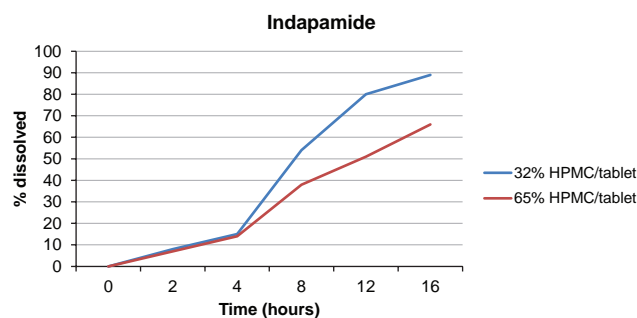


Fig. 5. Comparison of dissolution profile of indapamide modified release tablets containing different quantities of hydroxypropyl-methylcellulose

The discriminating power of the proposed dissolution method on different quantities of HPMC K4M PR was tested. The results confirm that our method has discriminating power on the excipients quantitative changes, too (Figure 5).

Regarding sample stability, indapamide remained stable in the dissolution medium for at least 16 hours after the end of each dissolution test. The concentration found after 16 hours storage was 97.3% of the initial drug amount.

### Conclusions

A discriminating dissolution method was developed for a practically insoluble drug such as indapamide. A screening study was conducted to optimize the dissolution parameters: the optimal dissolution medium and the optimal speed of rotation of the dissolution tester for the indapamide in the selected medium was chosen. A robust HPLC method for analyzing dissolution samples containing indapamide from modified release tablet form was developed and successfully validated. The dissolution test conditions should discriminate product changes that may affect the performance of the pharmaceutical product in vitro and in vivo. The discriminative power of the dissolution method

was demonstrated by preparing formulas with different types of hypromellose in the same ratio. The dissolution profiles between these formulas were compared. It can be observed that the technological changes affected the dissolution profiles, so the developed dissolution method can detect little changes in the composition.

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