

## Development and Validation of a Dissolution Method with UV Analysis for Coumarin Tablets

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### Abstract

A dissolution test for coumarin tables was developed and validated. To establish a dissolution procedure various trials have been done in different pH and in stirring speeds and drug release was analyzed by previously validated UV spectrophotometric method. After selection of the best conditions, the method was validated using USP Apparatus II (paddle), 75-rpm rotation speed, 900 mL of phosphate buffer pH 6.8, and test time of 45 min. Previously the developed analytical method was validated for accuracy, precision and recovery studies. Statistical analysis proved that the method was precise, reproducible, selective, specific, and accurate for the analysis of coumarin.

**Keywords:** Coumarin, dissolution, tablets, validation, spectrophotometer.

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### Introduction

Drug absorption from solid pharmaceutical dosage forms following oral administration depends on the stages of disintegration, de-aggregation and drug solubilisation from the pharmaceutical dosage form under physiological conditions, and the permeability across the gastrointestinal tract. Because of the critical nature of the first two steps, *in vitro* dissolution may be relevant to the prediction of *in vivo* performance (Abbou 1995, Emami 2006).

The use of dissolution testing as a quality control tool grew explosively in 1970s. During the 1980s the pharmaceutical industry began to develop a data base that connected the dissolution performance of oral solid dosage form. Dissolution testing can provide information not only on the rate and extent of drug absorption in the body but also on the effects of drug biopharmaceutical properties and formulation principles on the release properties of a pharmaceutical product (Lee et al. 2008). Therefore, *in vitro* dissolution tests are usually used to assess the lot-to-lot quality of a drug product, guide development of new formulations, and ensure continued product quality and performance after certain changes such as formulation, manufacturing process, site of manufacture, and the scale-up of the manufacturing process. The dissolution properties (extent and profile) of a finished dosage form should be monitored during product scale-up (Glen 1995, Lakshmana Prabu et al. 2009).

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A dissolution medium containing surfactant can better simulate the environment of the gastrointestinal tract than a medium containing organic solvents or other non-physiological substances, making the dissolution test conditions more useful in evaluating drug quality (Shah et al. 1989, Jinno et al. 2000).

The dissolution procedure requires apparatus, dissolution medium, and test conditions that provide a method that is discriminating yet sufficiently rugged and reproducible for day-to-day operation and capable of being transferred between laboratories (Dias et al. 2011). Hence there is a definite need for the development of an appropriate dissolution procedure; that underlines a powerful tool to evaluate the impact of formula, process, equipment and site changes that may occur during product scale-up; dissolution test has moved from a traditional quality control test to a substitute *in vitro* bioequivalence (BE) study (Amidon et al. 1989, Galia et al. 1998). The dissolution profile assessment of drug product must be quite definite and highly reproducible (Shah et al. 1997, Pillai et al. 2001).

Coumarin and other benzopyrones, such as 5, 6 benzopyrone, 1, 2 benzopyrone, diosmin and others are known to stimulate macrophages to degrade extracellular albumen, allowing faster Resorption of edematous fluids. Presently, there are no official monographs for tablet and no dissolution test has been described in literature for this drug. A universal dissolution medium made of affordable components guaranteed to generate an IVIVC will perhaps be identified in the future (Rohrs 2001). The objective of our present work is to establish a dissolution procedure for 200 mg coumarin in tablet pharmaceutical dosage form and to compare the dissolution profiles obtained from commercial product, to assess the efficacy of the dissolution methods.

*Dissolution test importance* (Moore and Flanner 1996)

- Optimization of therapeutic effectiveness during product development and stability assessment.
- Routine assessment of production quality to ensure uniformity between production lots.
- Assessment of 'bioequivalence', that is to say, production of the same biological availability from discrete batches of products from one or different manufacturers.
- Prediction of '*in vivo*' availability, i.e. bioavailability (where applicable).
- Although initially dissolution test has been developed for oral dosage forms, now it has been extended to various other forms such as topical and transdermal systems and suppositories in order to determine the drug release profile.

## **Materials and Methods**

### *Materials*

Coumarin reference standard and tablet were received as a gift samples from Pharm Products Pvt. Ltd. Thanjavur, Tamilnadu. Potassium dihydrogen phosphate and sodium hydroxide were procured from S.D. Fine Chemicals Mumbai. Methanol and hydrochloric acid were procured from Qualigens Fine Chemicals, Mumbai. Double-distilled water was used throughout the study.

### *Instruments*

The dissolution tester (model DA-6DR USP standard) equipped with six dissolution vessels; ultrasonic Bath (model 100H); Sartorius analytical balance (model CP-225D); Shimadzu UV/VIS visible spectrophotometer (model 1601); and Sartorius digital pH meter (model PB-11) were used.

A Shimadzu UV/Vis spectrophotometer model 1601 (Japan) was employed with spectral bandwidth of 0.1 nm and wavelength accuracy of  $\pm 0.5$  nm with automatic wavelength correction with a pair of 3 mm quartz cells were used for all the spectral and absorbance measurement. Commercially available tablets were procured from the local market.

#### *Dissolution testing condition*

To establish a universal dissolution procedure for coumarin 200 mg tablets phosphate buffer pH 6.8 and 7.4 were used. Drug release profile was carried out using basket (USP Apparatus 1) and paddle (USP Apparatus II) at different stirring speeds like 50, 75 and 100 rpm. The dissolution conditions are shown in Table 1. An aliquot sample of 5.0 mL was withdrawn at various time intervals like 15, 30, 45 and 60 minutes, and an equal volume of the fresh medium was replaced in order to maintain a constant total volume. Samples were filtered and suitably diluted with dissolution medium and analyzed spectrophotometrically at 277 nm; the procedure adopted in spectrophotometric method for analysis of coumarin was previously validated. The cumulative percentage of drug released was plotted against time, in order to obtain the release profile and to calculate the *in vitro* dissolution data ( $n=12$ ).

**Table 1.** Conditions for dissolution test optimization of coumarin in tablet pharmaceutical form

Trial	Apparatus	Dissolution Medium	Stirring Speed (rpm)
1	Paddle	Phosphate buffer pH 6.8	50
2	Paddle	Phosphate buffer pH 6.8	75
3	Paddle	Phosphate buffer pH 6.8	100
4	Paddle	Phosphate buffer pH 7.4	50
5	Paddle	Phosphate buffer pH 7.4	75
6	Paddle	Phosphate buffer pH 7.4	100
7	Basket	Phosphate buffer pH 6.8	50
8	Basket	Phosphate buffer pH 6.8	75
9	Basket	Phosphate buffer pH 6.8	100
10	Basket	Phosphate buffer pH 7.4	50
11	Basket	Phosphate buffer pH 7.4	75
12	Basket	Phosphate buffer pH 7.4	100

#### *Preparation of standard*

About 50 mg of coumarin was weighed accurately and transferred into two 100 ml volumetric flask separately. To the first flask about 50 ml phosphate buffer pH 7.4 was added and the contents were dissolved and diluted up to the mark with phosphate buffer pH 7.4; to the second flask about 50 ml phosphate buffer pH 6.8 was added and the contents were dissolved and diluted up to the mark with phosphate buffer pH 6.8 (500  $\mu\text{g/ml}$ ).

#### *Dissolution method validation*

Dissolution test validation was performed according to ICH requirements by means of the analysis of linearity, precision, selectivity and accuracy parameters.

#### *Preparation of calibration curve*

Different volumes of stock solutions were suitably diluted with corresponding dissolution medium (4, 6, 8, 10, 12 and 14  $\mu\text{g/ml}$ ) to get the desired concentrations. Each solution was analyzed in triplicate. The amplitude values were plotted against the corresponding concentrations to obtain the linear calibration curve.

### *Precision*

For the determination of intra-day and inter-day accuracy and precision of the assay, samples containing coumarin (6, 8 and 10 µg/ml) were analyzed for six times in a day (intra-day) and three consecutive days. Precision was calculated as inter-day and intra-day coefficient of variation.

### *Robustness of the method*

Small deliberate changes in the wavelength ( $\pm 5$  nm) were introduced and the effects on the results were examined.

### *Selectivity*

The selectivity of the method was assessed by analyzing standard drug, pharmaceutical product and placebo and comparing the  $\lambda_{\max}$  of the standard with that of the sample to determine whether the pharmaceutical product and placebo lead to interfere in the estimation.

### *Recovery studies*

The recovery studies of the method were assessed by spiking the standard coumarin with the pre-analyzed samples and the mixtures were analyzed by the proposed method. At each level of the amount, six determinations were performed. This was done to check the recovery of the drug at different levels in the formulation.

## **Results and Discussion**

Developing dissolution methods for a new product has been a consistent challenge for the pharmaceutical scientist. Understanding the physicochemical properties of the drug is crucial for determining the most effective strategy for enhancing dissolution. Dissolution testing should be carried out under physiological conditions, if possible, allowing interpretation of dissolution data with respect to the *in vivo* performance of a drug product.

Dissolution is an official test used by pharmacopoeias for drug evaluation release of solid and semisolid dosage forms, and it is routinely used in Quality Control (QC) and Research and Development (R and D). However, it is critical that dissolution methods developed for use as quality control (QC) tools consistently deliver reliable test results and assess drug product quality attributes (e.g., particle size, polymorphic form, or excipients). The purpose of *in vitro* dissolution studies in QC is batch to batch consistency and detection of manufacturing deviation while in R and D the focus is to provide some predictive estimate of the drug release in respect to the *in vivo* performance of a drug product.

The proposed analytical method for determination of coumarin in tablet formulation was found to be simple, accurate, economical and rapid. Coumarin exhibited maximum absorption at 277 nm and obeyed Beer's law in the concentration range of 4 to 12 µg/ml. The proposed method for determination of coumarin showed linear regression  $y = 0.075x - 0.001$  and  $0.0749x - 0.001$  with a correlation coefficient ( $R^2$ ) of 0.9990. The percentage recovery was found to be between 99.14 % and 99.50 % for both dissolution medium; which indicated the non-interference of the excipients which are present in the formulation for the determination of drug. The study was made to test ruggedness of the method through the inter-day and intra-day analysis of samples. Results obtained confirmed ruggedness of the method. The developed analytical methods were found to be accurate, precise, reproducible and stable, which indicated that this method can be used for the routine analysis of coumarin in bulk drug and its solid dosage form.

Definition of the appropriate parameters for coumarin tablets dissolution test was performed in phosphate buffer pH 6.8 and 7.4; using paddle and basket apparatus and various stirring

speeds like 50, 75 and 100 rpm. Samples were withdrawn at various time intervals and analyzed spectrophotometrically. As the release was lesser than 85 % within 60 minutes with Apparatus I at the stirring speeds of 50, 75 and 100 rpm in both the phosphate buffer media viz. 6.8 and 7.4, it was less likely to be accepted as satisfactory dissolution requirement. On the other hand, the use of paddle as apparatus at the stirring speed of 75 rpm gave a satisfactory dissolution of the drug in 45 minutes of the test, with drug release higher than 85%. The dissolution release results are shown in Figure 1 and 2.

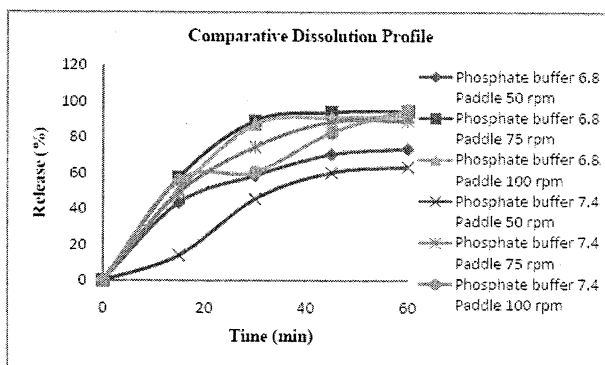


Figure 1. Comparative dissolution profile of Coumarin tablets in paddle method

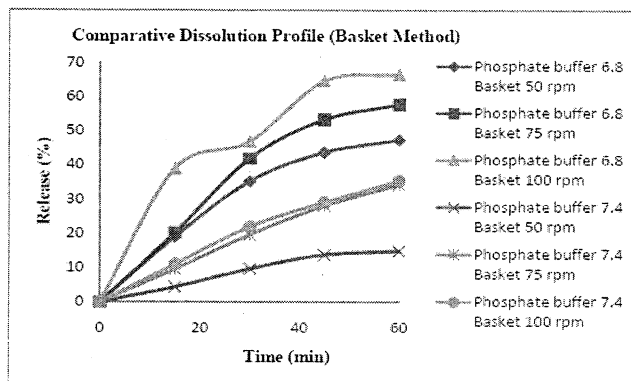


Figure 2. Comparative dissolution profile of Coumarin tablets in basket method

The variables phosphate buffer pH 6.8, rotation speed of dissolution medium at 50 rpm and basket as apparatus were defined as the low level parameters. The variables phosphate buffer pH 7.4, rotation speed of dissolution medium at 100 rpm and paddle as apparatus were defined as the high level parameters.

## Conclusion

The most selective condition for carrying out the dissolution assessment of coumarin tablets were: 900mL of phosphate4 buffer pH 6.8 at  $37 \pm 0.5^\circ\text{C}$  as dissolution medium, apparatus Type II (paddle method) at the stirring speed of 75 rpm and collect time in 45 minutes. The validation showed that the dissolution test is appropriate for quantification of coumarin in

tablet pharmaceutical form for *in vitro* studies, presenting selectivity, linearity, precision and accuracy.

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