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Research Article

ANALYTICAL METHOD DEVELOPMENT FOR DISSOLUTION RELEASE OF FINISHED SOLID ORAL DOSAGE FORMS

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ABSTRACT

Tablets are solid dosage forms containing medicinal substances with or without suitable diluents or excipients. To evaluate the quality of tablets several analytical parameters such as Dissolution release, Assay content and related substances analysis has to perform. In the present study analytical method development for dissolution of finished dosage forms are carried out by HPLC method. The dissolution release estimation of various types of tablets is performed and the results are reported. To establish the in-vitro and in-vivo correlation concepts the difference factor F1 and the similarity factor F2 has calculated against innovator sample. The multimedia dissolution at various time points were carried out in different pH such as pH 1.2,4.5,6.8,7.0 and 7.4 to match with innovator sample profile.[1] [2] [3]

Keywords: Analytical method development, Dissolution method development, Multimedia dissolution, Dissolution profile, Finished dosage forms, In-vitro release estimation

INTRODUCTION

The rate and extend in which the amount of drug substance dissolved over a period of time is called dissolution. It is expressed as percentage release of drug substances present dosage forms such Tablets, Capsules, oral suspensions and ointments. In our present study dissolution method development for various kinds of tablets such as immediate release or instant release, modified, Extended release and Enteric coated. [1] [2] [3]

The dissolution method developed for these kinds of tablets is compared with innovator's reference product to evaluate the release pattern and establish the method for estimating drug release by High Performance Liquid chromatography method.

ANALYTICAL METHOD DEVELOPMENT

Liquid chromatography (LC) is an analytical chromatographic technique that is useful for separating ions or molecules that are dissolved in a solvent. If the sample solution is in contact with a second due to difference in adsorption, ion exchange, partitioning or size. These differences allow the mixture of components to be separated from each other by using these differences to determine the transit time of the solute through a column.

Nature of the sample

- Number of Components present.
- Chemical structure of components.
- P_{Ka} values of components.
- UV spectra of components.
- Concentration range of components in sample of interest.
- Sample solubility.

Initial condition for Reverse Phase HPLC method development

Separation Variable	Preferred Initial Choice
Column packing	C-8 or C-18, less acidic silica
Column Configuration	15x0.45cm column, 5µ particles
Flow rate	1.5 - 2.0 ml/min
Mobile phase or	Acetonitrile, water (neutral samples)

Acetonitrile-buffer (ionic samples), buffer may be 25-50m.M Potassium Phosphate at pH 2-3 for the initial experiment, a 5-10% B gradient in 60 min is recommended.[3] [7] [9] [10]

Temperature	35-40ºC
Sample volume	< 25 µl

DISSOLUTION TESTING

Dissolution should form an essential part of pharmaceutical development of solid oral dosage forms and usually suspensions. The media and conditions chosen in the studies will depend on the required release characteristics of the intended product.

For immediate release products the paddle (Apparatus 2, usually at 50 to 75 rpm) and basket (apparatus 1, usually at 100 rpm) are conventional. Immediate release typically means that 75% of the API is dissolved within 45 minutes. Lately the terms rapidly dissolving (85% in 30 minutes) and very rapidly dissolving (85% in 15 minutes) become popular and important in dissolution testing.

The following media should be considered for immediate release products during development studies:

- pH 6.8 buffer (or simulated intestinal fluid without enzymes)
- pH 4.5 buffer
- pH 1.2 buffer (or simulated gastric fluid without enzymes) or 0.1 M hydrochloric acid.
- Water may be considered as an additional medium

For development purposes the generation of dissolution profiles at short intervals such as 10, 15, 20, 30 and 45 minutes in the above media are strongly recommended. This would enable:

- The selection of the formulation, by comparison of the dissolution profiles with that of the innovator product. This should be a basic strategy in pharmaceutical development to maximize the chances of bioequivalence.
- Comparison of the release properties of the pivotal batches to demonstrate invitro similarity, which is considered essential for retention of efficacy and safety. Note that bioequivalence studies are done normally only once on a pivotal batch during development. It must therefore be demonstrated that the product retain the release characteristics up to and during commercial production.
- The selection of the dissolution specifications (conditions and acceptance criteria) for product release and stability study purposes. A dissolution specification should be discriminating, implying that it should be able to detect inaquate release properties of the commercial batches.

- Post-approval amendment application. If the amendment is of a major nature and requires bioequivalence studies, invitro data may be acceptable, provided that (1) the profiles of the amendment batch and the current batch are similar and (2) that the dissolution study design is acceptable (preferably the three media and short interval multipoint as mentioned above).

Two scenarios for comparing the profiles obtained multipoint dissolution are operative:

1. If both the test and reference product show more than 85% dissolution within 15 minutes, the profiles are considered similar.

2. Calculate the f2 value. If f_2 >50 the profiles are normally regarded similar. Note that only one measurement should be considered after 85% dissolution of both products has occurred and excluding point zero.

In this equation f2 is the similarity factor, n is the number of time points, R(t) is the mean percent drug dissolved of e.g. a reference product, and T(t) is the mean percent drug dissolved of e.g. a test product.

The evaluation of similarity is based on the conditions of

- A minimum of three time points (zero excluded)
- 12 individual values for every time point for each formulation
- Not more than one mean value of > 85% dissolved for each formulation
- That the standard deviation of the mean of any product should be less than 10.% from second to last time point.

AIM OF THE PRESENT STUDY [8] [9] [10]

Dissolution test is part of general test specification of solid oral dosage forms (tablets and capsules), oily suspensions, etc. and same is carried out for following purposes.

a. To access batch to batch quality during formulation development and during regular production for batch release.

b. To assist bio-availability and bio-equivalence study. Wherever possible comparison of dissolution studies with these studies is also provided.

c. Dissolution test is also part of stability study to establish shelf life.

d. Dissolution data (3 or 5-media dissolution study) is required for getting new drug formulation approval from local and international authority.

e. To justify scale up and post approval changes (SUPAC) in dosage forms.

Depending upon requirement, single, double or multiple time point dissolution study and single or multiple media dissolution study shall be carried out.

The dissolution results shall be calculated in Microsoft Excel software. The data shall be reported on all the tablets or capsules. Range that includes minimum and maximum % dissolution from no. of tablets or capsules at each time point should be reported.

If required. Fl and F2 statistical calculations shall be performed on data obtained from innovator and formulation development sample. Conclusion regarding similarity and dissimilarity of dosage form shall be reported. .

In dissolution study, tablet or capsule formulation is placed in polypropylene jar of 1000 ml capacity containing 900 or 500ml of suitable dissolution medium depending upon label claim .The medium is allowed to rotate at certain speed of 50 to 100 RPM by means of a stainless steel rotator Called Paddle or Basket. The time points for dissolution for fixed single or multiple time intervals. After specified time, aliquot of samples are withdrawn and analyzed for the amount of drug dissolved in that medium. This indicates drug dissolution capacity in presence of excipients in that medium and thus can be used as quality tool during formulation development and manufacturing.

Following are the parameters that are usually considered during method development for Dissolution.

a. Selection of dissolution medium

b. Selection of apparatus and RPM (Speed)

c. Selection of dissolution time interval (single and multiple point)

d. Selection of other parameters like, media volume, temperature, etc.

Selection of dissolution medium

Selection of dissolution medium depends upon following parameters.

a. Type of formulation (Immediate or modified release).

b. Solubility characteristics of active component.

c. Type formulation design, e. g. Soft gel capsule, Hard gel capsule, Tablets, Oily suspension, etc.

Bio pharmaceutical Classification System (BCS) has classified drug solubility into four categories.

i. Highly soluble and highly permeable substances

ii. Poorly soluble and highly permeable substances

iii. Highly soluble and poorly permeable substances

iv. Poorly soluble and poorly permeable substances

For formulation having active substances that fall in Class-i and Class-iii categories, but are immediate release formulations (uncoated and film coated), 0.1N hydrochloric acid as dissolution medium shall be used. However if formulation of this class is modified release (delayed release, enteric coated and sustained release), in that case 6.8 pH buffer shall be used as dissolution medium. In both the cases, pH 4.5 buffer shall be intermediate media for dissolution profile.

For formulation having active substances that fall in Class-ii and Class-iv categories, but are immediate release formulations of uncoated and film coated tablets, 6.8 buffer as dissolution medium shall be used. For modified release formulation (delayed release, enteric coated and sustained release) of this class, 6.8 pH buffer shall be used as dissolution medium. In both the cases, pH 4.5 buffer shall be intermediate media for dissolution profile.

For formulation of Class-ii and Class-iv category, because of poor solubility of active drug, it I may require to use a surfactant (i.e. Sodium lauryl sulphate) to enhance drug solubility in dissolution media. Concentration of surfactant can be from 0.5 to 2.0%. Higher concentration other than this should be justified.

For formulation of Class-ii and Class-iv category, if all the medias are tried out (0.1 S HCI, pH-6.8, use of surfactant) and if dissolution rate is not satisfactory in required time, in that case Tris buffer pH-9.0as dissolution media shall be used. In such cases justification along with various media trial results should be provided.

For all formulations, if dissolution test is applicable as per guidelines requirement the dissolution test should be provided. In case test is not provided due to any reason, same should be justified and information taken on efforts taken to develop the same should be provided.

For regulatory submission purposes and for comparison with innovators. We also have to carry out dissolution study in additional dissolution media like pH-7.5 buffer (Apart from 0.1 N HCI, pH-6.8, pH-4.5 and water). To compare data with innovators, statistical parameters like Fl and F2 calculated on our and innovators dissolution data is very useful and recommended.

Selection of RPM

The selection of RPM depends upon type of formulation, solubility characteristics of active substances and apparatus used for dissolution study.

For capsules (both soft gel and hard gel), USP-I i. e. Basket apparatus is recommended, rotation speed for paddle shall be 50 to 75 RPM. Use of higher RPM other than this should be justified.

For tablets, USP-II i. e. paddle apparatus is recommended, rotation speed for basket shall be 75 to 100 RPM. Use of higher RPM other than this should be justified.

Any change in apparatus and RPM other than recommended parameters should be justified.

Other apparatus that are mentioned in USP general chapters (e. g. Flow through cell) shall be used for specific formulation.

Selection of dissolution time intervel (single and multiple point)

Dissolution time is defined as the time in minutes at which maximum amount (+80% of label claim) of drug is dissolved.

For immediate release dosage forms, dissolution time from 30min. to 60min. is recommended. In some cases dissolution time may be higher i. e. up to 90min. to l20min. In such cases suitable justification should be provided.

For modified release formulations (delayed release, enteric coated and sustained release), time depends upon design of formulation, site of action and therapeutic use. Time for such formulations may be from about 6 hrs. to 24 hrs. or may be higher.

In most of the cases, (except in modified release formulations) single point dissolution analysis is sufficient. However in some cases two time point dissolution analysis may be required.

The % release of active components should meet the specification within specified time.

Selection of other parameters like, media volume, temperature, etc.

The volume of dissolution media is ideally 900ml, however if label claim is less than 5mg and if active substances has less absorbance at selected wavelength, then in that case dissolution volume can be reduced to 500ml.

The dissolution media temperature is fixed i. e. 37.0 (±0.5)ºC

Before starting dissolution analysis, ensure dissolution apparatus passes calibration test that can be performed periodically to ensure accuracy and precision of the same. Other factors that are to be checked are centeric position of rotator, wobble test, etc.

Before starting dissolution analysis, ensure that the dissolution media is properly degassed, especially in case of surfactants are used for dissolution media preparation.

Care should be taken during dissolution of tablets and soft gel or hard gel capsule (if paddle apparatus is used) that the same will not float on media surface. In such cases sinker of suitable size can be used.

Clean the dissolution apparatus and jars with suitable detergent, but do not wash with organic solvent.

In addition, IR solid oral dosage forms are categorized as having rapid or slow dissolution. Within this framework, when certain criteria are met, the BCS can be used as a drug development tool to help sponsors justify requests for biowaivers.

The following Immediate release, Sustained release and enteric coated tablets such as Ivabradine Hydrochloride, Metformine Hydrochloride, Olmesartan Medoxomil and Prednisolone tablets areselected respectively for the study of dissolution method development.

The developed method is compared with Innovators reference product and the Difference factor (F1), Similarity factor (F2) has evaluated with each formulation.

IVABRADINE HYDROCHLORIDE IMMEDIATE RELEASE TABLETS DISSOLUTION

Apparatus	: Apparatus II of BP (Paddle)
Medium	: 0.1M Hydrochloric acid ; 900 ml
Time	: 5,10,15,20,30 &45 minutes
Speed	: 50 RPM
Temperature	: 37° C ± 0.5° C

Standard Preparation

Weigh accurately about 0.041gm of Ivabradine HCl WS in to a 100ml volumetric flask, add 30ml of diluent, shake and sonicate to dissolve the content and make up the volume with diluent. Transfer 2 ml of the above solution to 100 ml volumetric flask and make up the volume with dissolution medium Filter the solution through membrane filter.[1] [3] [9]

Sample Preparation

Set the apparatus at above condition and place one tablet each in 6-dissolution bowl. Run the apparatus for 45 minutes. Withdraw 10 ml of the sample in the above time intervals from each bowl, replacing the same amount every time with dissolution medium. Filter the solution through membrane filter.

CHROMATOGRAPHIC CONDITION

Apparatus: HPLC

Column: C18, 200 x 4.6 mm; 5µm; SS Column (Inertsil ODS)

Wave length: 235 nm

Flow rate : 1.5 ml/min.

Inj. Volume: 20µl

Column oven: 25°C

Mobile Phase: Filtered and degassed mixture of Buffer and Acetonitrile in the ratio of 700: 300

Buffer: Dissolve 6.8 gm of Sodium dihydrogen ortho phosphate in 1000 ml of water and adjust the pH 4.0 with dilute Ortho phosphoric acid

Diluent: Acetonitrile and Water in the ratio of 600:400

SYSTEM SUITABILITY

Chromatograph the Standard preparation and record the peak responses as directed under procedure. The theoretical plates for Ivabradine HCl peak should not be less than 2000, tailing factor should not be more than 2.0 and the relative standard deviation for 5 replicate injections should not be more than 2.0%.

PROCEDURE

Separately inject 10 microlitres of filtered portion of the Sample preparation and Standard preparation into the Chromatograph. Record the chromatogram and measure the responses for the major peak. Calculate the release in percentage with respect to label claim by using the following expression.

CALCULATION

$$\frac{AT}{AS} \times \frac{WS}{100} \times \frac{2}{100} \times \frac{900}{1} \times \frac{P}{100} \times 1 \times 1000 \times \frac{100}{5} \times \frac{468.59}{505.05}$$

=_____ % release of Ivabradine

Where `AS' is the Average area of Ivabradine HCl peak in Standard preparations;'AT' is the individual area of Ivabradine HCl peak in sample preparation`WS' is the Weight of Ivabradine HCl WS taken for Standard preparation in gm; 'P' is percent purity of Ivabradine HCl WS on as such basis; 468.59 is the molecular weight of Ivabradine and 505.05 is the molecular weight of Ivabradine HCl respectively.

Table 1: Innovator's reference product vs Test sample in pH1.2 medium

Time (t) [mins]	Reference ®	Test (T)	Rt-Tt	$(Rt-Tt)^2$	|Rt-Tt|
0	0.00	0.00	0.00	0.00	0.00
10	85.22	90.70	-5.48	30.03	5.48
20	100.11	100.40	-0.29	0.08	0.29
30	103.10	100.70	2.40	5.76	2.40
45	104.24	101.10	3.14	9.86	3.14
	392.67			45.73	11.31
Difference Factor - F1 [Acceptance Criteria : 0 - 15]					2.88
Similarity Factor - F2 [Acceptance Criteria : 50 - 100]					72.64

METFORMINE HYDROCHLORIDE SUSTAINED RELEASE TABLETS DISSOLUTION

Apparatus	Paddle
Medium	Phosphate Buffer pH 6.8, 900 ml
Time	1,2,3,4,6,8 & 10 hrs.
Speed	100 rpm
Temperature	37° C ± 0.5 ° C

Preparation of Phosphate buffer pH 6.8

0.68% w/v solution of Potassium dihydrogen orthophosphate adjusted to pH 6.8 ± 0.05% by the addition of 1M sodium hydroxide.

Standard Preparation

Weigh accurately about 0.055gm of Metformine HCl WS in to a 100ml volumetric flask, add 30ml of diluent, shake and sonicate to dissolve the content and make up the volume with diluent. Transfer 5 ml of the above solution to 50 ml volumetric flask and make up the volume with dissolution medium Filter the solution through membrane filter.[1] [3] [9]

Sample preparation

Transfer 900 ml of phosphate buffer pH 6.8 into each of the six vessels. Assemble the apparatus with paddle; warm the medium at 37°C ± 0.5°C.Place one tablet in each of six bowl and rotate the paddle for 45 minutes. Withdraw the sample of 20 ml from the dissolution vessels at the prescribed time point from a position midway between the surface of the dissolution medium and the top of the paddle and not less than 10 mm from the vessel wall. Filter 20 ml of the sample and reject first 5 ml of the filtrate, dilute 10 ml of the filtrate to 100 ml with water and further dilute 10 ml of the resulting solution to 100 ml with water.

Procedure

Determine the amount of C4H11N5·HCl dissolved by UV absorption at the wavelength of maximum absorbance at about 232 nm on portions of the solution under test passed through a 0.45-µm hydrophilic polyethylene filter, suitably diluted with Medium.

Calculate the amount of metformin hydrochloride (C4H11N5·HCl), in percentage, released at each time point by the following expression.

CALCULATION

$$\frac{AT}{AS} \times \frac{WS}{100} \times \frac{5}{50} \times \frac{900}{1} \times \frac{10}{100} \times \frac{P}{100} \times 1 \times 1000 \times \frac{100}{500}$$

=_____ % release of Metformine HCl

Where `AS' is the Average area of Metformine HCl; peak in Standard preparations;'AT' is the individual area of Metformine HCl; peak in sample preparation`WS' is the Weight of Metformine HCl WS taken for Standard preparation in gm; 'P' is percent purity of Metformine HCl; WS on as such basis.

Table 2: Innovator's reference product vs Test sample in pH6.8 medium

Time (t) [Hours]	Reference ®	Test (T)	Rt-Tt	$(Rt-Tt)^2$	|Rt-Tt|
0	0.00	0.00	0.00	0.00	0.00
1	27.90	30.33	-2.43	5.90	2.43
2	41.72	45.76	-4.04	16.32	4.04
3	53.72	57.66	-3.94	15.52	3.94
4	62.37	66.93	-4.56	20.79	4.56
6	74.32	79.92	-5.60	31.36	5.60
8	82.56	89.06	-6.50	42.25	6.50
10	87.83	93.71	-5.88	34.57	5.88
Average				166.73	32.95
Difference Factor - F1 [Acceptance Criteria : 0 - 15]					7.66
Similarity Factor - F2 [Acceptance Criteria : 50 - 100]					65.13

OLMESARTAN MEDOXOMIL TABLETS DISSOLUTION

Apparatus	: Apparatus II of BP (Paddle)
Medium	: 0.1M Hydrochloric acid, 900 ml
Time	: 45 minutes
Speed	: 75 RPM
Temperature	: 37° C ± 0.5 ° C

Preparation of 0.1M Hydrochloric acid

Mix 8.5 ml of hydrochloric acid in 1000 ml purified water.

Standard Preparation

Transfer about 0.022 g of Olmesartan Medoxomil WS, accurately weighed into a 100 ml volumetric flask, add 70 ml of diluent, sonicate to dissolve the contents and make up the volume with diluent. Dilute 5 ml of the above solution to 50 ml with dissolution medium.Filter the solution through 0.45µ filter. .[1] [3] [9]

Sample Preparation

Set the apparatus at above condition and place one tablet each in 6-dissolution bowl. Run the apparatus for 10 hours. Withdraw 10 ml of the sample in the above time intervals from each bowl, replacing the same amount every time with dissolution medium. Filter the solution through membrane filter.

CHROMATOGRAPHIC CONDITION

Apparatus: HPLC

Column: Cyano 250 X 4.6 mm, 5 µm

Flow rate: 1.0 ml/min

Detector: UV, 225nm

Mobile phase: Filtered and degassed mixture of Buffer and Acetonitrile in the ratio of 400:600.

Buffer: Dissolve 2.72gm of Sodium dihydrogen ortho phosphate in 1000 ml water and add 1 ml of Triethylamine.Then adjust the pH to 3.3 with Orthophosphoric acid

Diluent: Filtered and degassed mixture of Acetonitrile and water in the ratio of 80:20

SYSTEM SUITABILITY

Chromatograph the Standard preparation and record the peak responses as directed under procedure. The theoretical plates for Olmesartan Medoxomil peak should no be less than 2000, tailing factor should not be more than 2.0 and the relative standard deviation for 5 replicate injections should not be more than 2.0%.

PROCEDURE

Separately inject 20 microlitres of filtered portion of the Sample preparation and Standard preparation into the Chromatograph. Record the chromatogram and measure the responses for the major peak. Calculate the release in percentage with respect to label claim by using the following expression.

CALCULATION

$$\frac{AT}{AS} = \frac{WS}{100} \times \frac{5}{50} \times \frac{900}{1} \times \frac{P}{100} \times 1 \times \frac{100}{1000} \times \frac{}{20}$$

= ___ % release of Olmesartan Medoxomil

Where 'AS' is the average area of the Olmesartan Medoxomil peak in Standard preparation and 'AT' is the area of the Olmesartan Medoxomil peak in sample preparations, 'WS' is weight of Olmesartan Medoxomil WS taken for standard preparation in gm, 'P' is percent purity of Olmesartan Medoxomil WS on as such basis.

Table 3: Innovator's reference product vs Test sample in pH1.2 medium

Time (t) [mins]	Reference ®	Test (T)	Rt-Tt	$(Rt-Tt)^2$	|Rt-Tt|
0	0.00	0.00	0.00	0.00	0.00
10	97.47	93.31	4.16	17.29	4.16
20	98.00	95.87	2.13	4.54	2.13
30	99.13	99.68	-0.55	0.30	0.55
45	100.53	100.67	-0.14	0.02	0.14
Average	395.13			22.15	6.98
	Difference Factor - F1 [Acceptance Criteria : 0 - 15]				1.77
	Similarity Factor - F2 [Acceptance Criteria : 50 - 100]				79.62

PREDNISOLONE ENTERIC COATED TABLETS DISSOLUTION

Test - A

Dissolution Apparatus type	: Basket (Type I BP)
Medium	**:** 0.1 M hydrochloric acid
Volume	: 1000 ml
Time	: 120 minutes
Speed	: 100 RPM
Temperature	: 37° C ± 0.5° C

Test B

Dissolution Apparatus type	: Basket (Type I BP)
Medium	: Mixed Phosphate Buffer pH 6.8
Volume	: 900 ml
Time	: 45 minutes
Speed	: 100 RPM
Temperature	: 37° C ± 0.5° C

Operate the Dissolution method as per the dissolution conditions by using 0.1 M hydrochloric acid as the liquid. Operate the apparatus for 60 minutes under Test-A, introduce one tablet into each of six vessels.

Withdraw sample of 20 ml from the dissolution vessels at the prescribed time point from a position midway between the surface of the dissolution medium and the top of the basket and not less than 10 mm from the vessel wall.

Filtered through a nylon membrane filter having a nominal pore size not greater than 0.45 µm, discarding the first 10 ml of filtrate. Use the filtered sample as such.

No tablet shows signs of either disintegration or cracks that would allow the escape of the contents. Calculate the content of dissolved Prednisolone in percentage by using calculation (B)

Withdraw tablets and proceed immediately as directed under Dissolution Test-B

Preparation of Mixed phosphate buffer pH 6.8

Dissolve 28.80 g of disodium hydrogen orthophosphate (Na_2HPO_4, $12H_2O$, Molecular weight = 358.1) and 11.45 g of potassium dihydrogen orthophosphate (KH_2PO_4, Molecular weight = 136.1) in sufficient water to produce 1000 ml and adjust pH to 6.8 ± 0.05 and degas.

Perform Test B for same tablet by replacing 0.1M HCl by 900ml Mixed Phosphate Buffer pH 6.8 and perform the dissolution as per Test-B conditions

Solution (1): Accurately weigh and transfer about 50 mg of Prednisolone CRS / WS in a mixture of 42 volumes of water and 58 volumes of methanol (0.05% w/v solution) in a 100 ml volumetric flask dissolve and dilute to volume, dilute 5.0 ml to 100.0 ml with dissolution medium and further dilute 5.0 ml to 50.0 ml with dissolution medium to contain 0.00025 % w/v.

Solution (2): Transfer 900 ml of degassed solution of Mixed phosphate buffer pH 6.8 into each of the six vessels of the basket apparatus. Assembled the apparatus, warm the medium at 37°C ± 0.5°C

Withdraw sample of 20 ml from the dissolution vessels at the prescribed time point in Test-B from a position midway between the surface of the dissolution medium and the top of the basket and not less than 10 mm from the vessel wall.

Filtered through a nylon membrane filter having a nominal pore size not greater than 0.45 µm, discarding the first 10 ml of filtrate. Use the filtered sample as such (About 0.00025 % w/v of prednisolone.)

Withdraw sample of 20 ml from the dissolution vessels at the prescribed time point from a position midway between the surface of the dissolution medium and the top of the basket and not less than 10 mm from the vessel wall.

Filtered through a nylon membrane filter having a nominal pore size not greater than 0.45 µm, discarding the first 10 ml of filtrate. Dilute 5.0 ml. of the filtrate to 10.0 ml. with dissolution medium. (About 0.00025 % w/v of prednisolone.)

Analytical Conditions

Apparatus:	HPLC
Column:	Stainless steel column (20 cm X 4.6 mm) packed with octadecylsilyl silica gel for chromatography (10 µm), (Spherisorb ODS 1 is suitable or equivalent.
Detector:	UV, 254nm wavelength
Flow rate:	1ml/min
Injection volume:	20 µl

Mobile phase: A mixture of 42 volumes of water and 58 volumes of methanol

Procedure: Equilibrate the column, then inject 20 µl of blank, solution (1) five times and the solution (2) are injected into the chromatograph, the chromatograms are recorded and the peak responses are measured.

System suitability requirements: The test is not valid unless the column efficiency, determined using the peak due to prednisolone in the chromatogram obtained with solution (1), is greater than 15,000 theoretical plates per meter, Relative standard deviation, not more than 2.0.

Calculation (B): Calculate the content of dissolved Prednisolone in percentage from the following expression: [10] [12] [13]

$$\frac{AT}{AS} \times \frac{WS}{100} \times \frac{2}{100} \times \frac{900}{1} \times \frac{P}{100} \times 1 \times 1000 \times \frac{100}{5} \times \frac{468.59}{505.05}$$

= ___ % release of Prednisolone

Where:

A_T = Area of Prednisolone peak obtained in the Solution (2).

A_S = Average area of five injections of Prednisolone peak obtained with Solution (1).

W_S = Weight of the Prednisolone standard in mg.

P = Percent purity of Prednisolone standard on as such basis.

Table 4: Innovator's reference product vs Test sample in pH6.8 medium

Time (t) [mins]	Reference ®	Test (T)	Rt-Tt	$(Rt-Tt)^2$	|Rt-Tt|
0	0.00	0.00	0.00	0.00	0.00
10	86.72	88.52	-1.80	3.24	1.80
20	93.32	95.17	-1.85	3.42	1.85
30	95.36	98.31	-2.95	8.70	2.95
45	96.30	100.12	-3.82	14.59	3.82
Average	371.70			29.96	10.42
Difference Factor - F1 [Acceptance Criteria : 0 - 15]					2.80
Similarity Factor - F2 [Acceptance Criteria : 50 - 100]					76.78

RESULTS AND DISCUSSIONS

The dissolution method for various kinds of formulation has developed and the dissolution release against innovator reference products has performed. The evaluation of reference product against test product for each formulation in the release medium has performed. The difference factor (F1) , similarity factor (F2) has calculated against reference product release from each kind of formulation. The final results are find satisfactory well within the acceptance criterion and hence the developed method can be used for routine analysis for the estimation dissolution release in the finished dosage forms.

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