**RESEARCH ARTICLE**

# UV Spectroscopy Assay Method Development and Validation of Dimethyl Fumarate and Cyclosporine Drugs in Nano Dosage Forms

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**ABSTRACT**

An easy, precise and accurate spectroscopic technique for the estimation of cyclosporine and dimethylfumarate in pure form and nano dosage form has been developed. The proposed method involves dissolving dimethylfumarate in distilled water and cyclosporine in ethanol and subjecting resulting solution to UV spectroscopic assessment. Absorption maximum was found 210 nm and 214 nm respectively. Beer's law was obeyed in the concentration range of 1-5µg/ml and 1-8 µg/ml for dimethylfumarate and cyclosporine. Calibration curve showd linearity between absorbance and concentration as per line equation with $R^2$ value near 1. Validation was performed as ICH guildelines for linearity, accuracy, precision, Robustness, System suitability.

**Keywords:** Cyclosporine, Dimethyl fumarate, Spectrophotometry, Validation.

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**Source of support:** Nil

**Conflict of interest:** None

## INTRODUCTION

Dimethyl Fumarate (DMF) has anti inflammatory, Immunomodulatory property which is works on the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) pathway is involved in the cellular response to oxidative stress.

This pathway is activated by dimethyl fumarate in vitro and in vivo. In vitro specifically, it acts as a nicotinic acid receptor agonist. However, the exact mechanism of the effect in multiple sclerosis is unknown. It is believed to be through the Nrf2 pathway due to its anti-inflammatory and cytoprotective properties. Dimethyl fumarate is highly water soluble. All pharmacokinetic analysis was performed using monomethyl fumarate, the active metabolite of dimethyl fumarate due to the rapid pre-systemic hydrolysis by esterases. Dimethyl fumarate is extensively metabolized by esterases via hydrolysis into its active metabolite, monomethyl fumarate (MMF) before it reaches systemic circulation. The main sites of this metabolism include the gastrointestinal tract, blood, and tissues. MMF is further metabolized through the tricarboxylic acid cycle. The major metabolites in the plasma are MMF, fumaric acid, citric acid, and glucose. The cytochrome P450 system is not involved in the metabolism of dimethyl fumarate. The primary route of elimination is respiratory (60%) via exhalation of carbon dioxide. Other routes of elimination include renal (16%) and feces (1%). Trace amounts of unchanged MMF are present in the urine. Dimethyl fumarate has been found to be an allergic sensitizer at very low concentrations, producing eczema that is difficult to treat. Concentrations as low as 1 ppm may produce allergic reactions.[2,3]

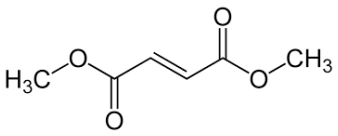


**Figure 1.1:** Chemical structure of dimethyl fumarate[1]

Cyclosporine is anti-inflammatory, immunomodulatory property. The most important effect of cyclosporine is to lower the activity of T cells and their immune response.

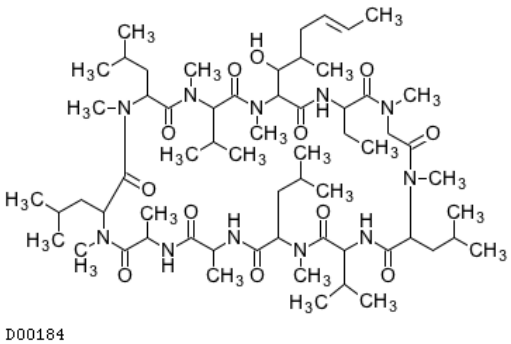


**Figure 1.2:** Chemical structure of cyclosporine A[4]

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It does this by binding to cyclophilin, a multifunctional protein that facilitates protein-folding, acts as a protein chaperone, and regulates the activity of other proteins. The resulting ciclosporin/cyclophilin complex inhibits the phosphatase activity of calcineurin which in turn is required for the activation of transcription factors that up regulate the expression of inflammatory cytokines. It is soluble in ethanol. The bioavailability of cyclosporine is variable, ranging from less than 10% to 89% in various populations. Because of its lipophilicity, cyclosporine is largely distributed outside of the blood volume once it is absorbed. Cyclosporine is primarily metabolized through the cytochrome P-450 Ill-A enzyme system in the liver, intestine, and kidney. At least 25 metabolites have been identified; however, the biologic activity and toxicity of these metabolites are considerably less than those of the parent compound are. Both cyclosporine and its metabolites are excreted primarily in the bile, with only 6% of the dose eliminated in the urine. Hepatic extensively metabolized by the cytochrome P450 3A enzyme system in the liver. It is also metabolized in the gastrointestinal tract and kidney to a lesser degree. The metabolites are significantly less potent than the parent compound. The major metabolites (M1, M9, and M4N) result from oxidation at the 1-beta, 9-gamma, and 4-N-demethylated positions, respectively. Elimination is primarily biliary with only 6% of the dose (parent drug and metabolites) excreted in the urine. Only 0.1% of the dose is excreted in the urine as unchanged drug. ADRs can include enlargement of the gums, convulsions, peptic ulcers, pancreatitis, fever, vomiting, diarrhea, confusion, hypercholesterolemia, dyspnea, numbness and tingling particularly of the lips, pruritus, high blood pressure, potassium retention possibly leading to hyperkalemia, kidney and liver dysfunction (nephrotoxicity and hepatotoxicity), burning sensations at finger tips, and an increased vulnerability to opportunistic fungal and viral infections. It is listed as IARC Group 1 carcinogens (sufficient evidence of carcinogenicity in humans).[5]

## MATERIALS AND METHODS

### Chemicals and Reagents

Dimethyl fumarate pharmaceutical grade were kindly supplied as gift sample by adventus laboratories pvt. Ltd. Makarpura, Vadodara. Cyclosporine pharmaceutical grade were kindly supplied as gift sample by Concord Biotech Limited, Ahmedabad, Gujarat. Distilled water was supplied from parul university, Vadodara. Ethanol was supplied from Shree Chalthan Vibhagkhand, Surat. UV Spectrometer (1800 240 V Shimadzu corp.) used for drug analysis.

### Analytical Method Development for Dimethyl Fumarate and Cyclosporine

The analytical method for dimethyl Fumarate and cyclosporine was developed using UV spectroscopy method.

### Calibration curve of Dimethyl Fumarate in Water and Phosphate Buffer pH 5.5 (PBS pH 5.5)

Calibration curve of dimethyl Fumarate was developed in distilled water and phosphate buffer pH 5.5.

*Preparation of stock solution in distilled water*

A 5 mg of dimethyl fumarate was weighed accurately and transferred to 50 mL volumetric flask. Accurately, 10 mL of distilled water was added to dissolve it and volume was made up to 50 mL with distilled water.[6,7]

*Preparation of calibration curve*

The above stock solution was scanned for the maximum absorbance using UV visible double beam spectrophotometer. The $\lambda_{max}$ of dimethyl fumarate in distilled water was found to be 210 nm. The above stock solution (100 µg/mL) was further diluted to get concentration of in the range of 2-10 µg/mL. Absorbance of each solution was measured using UV-Vis double beam spectrophotometer by putting reference standard of respective medium. The standard curve was generated for entire range of concentrations and the experiment was performed in triplicate.

*Preparation of stock solution in Phosphate Buffer pH 5.5*[8]

A 5 mg of dimethyl fumarate was weighed accurately and transferred to 50 mL volumetric flask. Accurately 10 mL of PBS pH 5.5 was added to dissolve it and volume was made up to 50 mL with water.

*Preparation of calibration curve*

The above stock solution was scanned for the maximum absorbance using UV visible double beam spectrophotometer. The $\lambda_{max}$ of dimethyl fumarate in PBS pH 5.5 was found to be 210 nm. The above stock solution (100 µg/mL) was further diluted to get concentration of in the range of 1-5 µg/mL. Absorbance of each solution was measured using UV-Vis double beam spectrophotometer by putting reference standard of respective medium. The standard curve was generated for entire range of concentrations and the experiment was performed in triplicate.

*Calibration Curve of Cyclosporine A in Ethanol*[9]

Calibration curve of cyclosporine was developed in Ethanol.

*Preparation of Stock solution of cyclosporine A in Ethanol*

A 5 mg of cyclosporine *A* was weighed accurately and transferred to 50 mL volumetric flask. Accurately, 10 mL of Ethanol was added to dissolve it and volume was made up to 50 mL with Ethanol.

*Preparation of calibration curve*

The above stock solution was scanned for the maximum absorbance using UV visible double beam spectrophotometer. The $\lambda_{max}$ of cyclosporine in ethanol was found to be 214 nm. The above stock solution (100 µg/mL) was further diluted to get concentration of in the range of 1-8 µg/mL. Absorbance of each solution was measured using UV-Vis double beam spectrophotometer by putting reference standard of respective medium. The standard curve was generated for entire range of concentrations and the experiment was performed in triplicate.

### Analytical method Validation was carried out for Dimethyl fumarate Drug and Cyclosporine A drug[10,11]

The proposed method was validated in compliance with ICH Guidelines for Linearity, Accuracy, Precision, Specificity,

Robustness, System Suitability parameters by the following procedures.

*Preparation of Standard and Sample solution of Dimethyl fumarate drug*

Weighed accurately 5 mg dimethylfumarate drug, added in 50 mL volumetric flask and dissolved in 10 mL distilled water then 45 mL distilled water was added to adjust the volume up to 50 mL (100 µg/mL).

*Preparation of Standard and Sample solution of Cyclosporine drug*

Weighed accurately 5 mg cyclosporine A drug, added in 50 mL volumetric flask and dissolved in 10 mL ethanol then 45 mL ethanol was added to adjust the volume upto 50 mL (100 µg/mL).

*Selection of wavelength*

Scan the standard solution in UV spectrophotometer between 200 nm to 400 nm on spectrum mode, using distilled water which shown in Figure 1.3 Blank spectrum of distilled water and ethanol as a blank which shown in Figure 1.4. The λmax of dimethyl Fumarate spectrum which shown in Figure 1.5 and cyclosporine are shown in Figures 1.6 respectively.

**Linearity**

Linearity for dimethylfumarate in distilled water is shown in Table 1.1 and in phosphate buffer is shown in Table 1.2. Calibration curves of dimethyl fumarate in distilled water is shown in Figure 1.7 and dimethyl fumarate in phosphate buffer

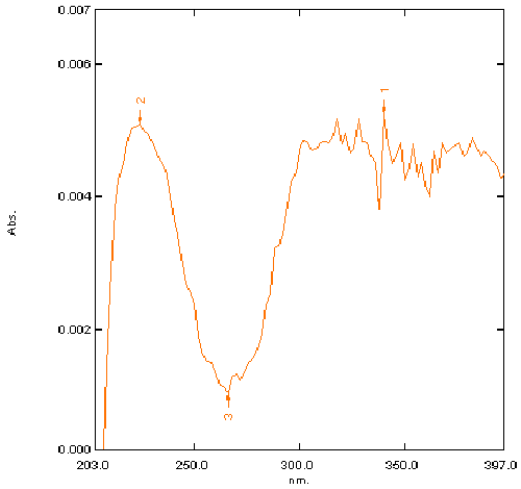


**Figure 1.3:** Blank Spectrum of Water

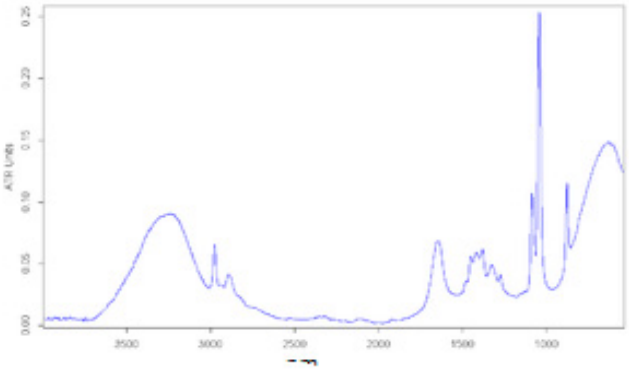

**Figure 1.4:** Blank Spectrum of Ethanol

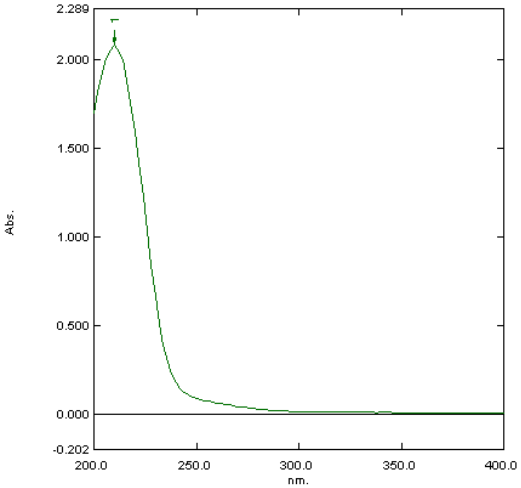


**Figure 1.5:** The lamda max of dimethylfumarate spectrum

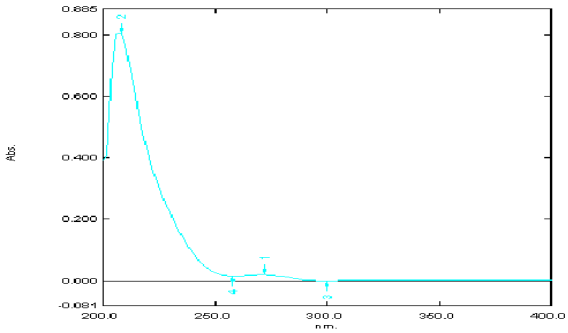


**Figure 1.6:** The lamda max of dimethylfumarate spectrum

**Table 1.1:** Calibration curve of Dimethyl Fumarate[12,13] in distilled water

| *SR No.* | *Concentration (µg/ml)* | *Mean Absorbance (±) SD* |
|---|---|---|
| 1 | 2 | 0.209±0.01 |
| 2 | 4 | 0.357±0.01 |
| 3 | 6 | 0.554±0.02 |
| 4 | 8 | 0.692±0.02 |
| 5 | 10 | 0.871±0.01 |

(Wheren=3, Mean± SD)

**Table 1.2:** Calibration curve of Dimethyl Fumarate in Phosphate Buffer pH 5.5

| *SR No.* | *Concentration (µg/ml)* | *Mean Absorbance (±) SD* |
|---|---|---|
| 1 | 1 | 0.202±0.01 |
| 2 | 2 | 0.332±0.01 |
| 3 | 3 | 0.482±0.02 |
| 4 | 4 | 0.652±0.02 |
| 5 | 5 | 0.791±0.01 |

(Wheren=3, Mean± SD)

**Table 1.3:** Accuracy results

| *% Level* | *Replicates* | *Concentration of standard solution (µg/ml)* | *Concentration of sample solution (µg/ml)* | *Absorbance of Standard* | *Absorbance of Sample* | *% recovery* |
|---|---|---|---|---|---|---|
| | I | 10 | 10 | 0.882 | 0.885 | 99.66 |
| 80% | II | 10 | 10 | 0.880 | 0.884 | 99.54 |
| | III | 10 | 10 | 0.883 | 0.882 | 100.11 |
| | I | 10 | 10 | 0.663 | 0.664 | 99.84 |
| 100% | II | 10 | 10 | 0.662 | 0.665 | 99.54 |
| | III | 10 | 10 | 0.660 | 0.664 | 99.39 |
| | I | 1 | 1 | 0.451 | 0.453 | 99.56 |
| 120% | II | 1 | 1 | 0.450 | 0.455 | 98.90 |
| | III | 1 | 1 | 0.452 | 0.454 | 99.55 |

is shown in Figure 1.8 respectively.

Linearity of cyclosporine in ethanol is shown in Table 1.9. Calibration Curve of cyclosporine in ethanol is shown in Figure 1.9.

**Accuracy**

Preparation of standard stock solution of dimethyl fumarate and cyclosporine.

*Preparation of standard and sample mixture*

- *Level I (80%)*

Volume of 0.5 mL sample stock solution, 0.3 mL of standard solution was transferred to 10 mL volumetric flask and volume was made up to mark with diluent (three replicates).

- *Level II (100%)*

Volume of 0.5 mL sample stock solution, 0.5 mL working standard stock solution was transferred to 10 mL volumetric flask and volume was made up to mark with diluent (three replicates).

- *Level III (120%)*

Volume of 0.5 mL sample stock solution, 0.7 mL of working standard stock solution was transferred to 10 mL volumetric flask and volume was made up to mark with diluent (three replicates).

**Determination**

The resulting mixture was injected repeatedly into the UV visible spectrophotometer, the absorbance was recorded and the % recovery of standard DMF and cyclosporine was calculated. The results obtained were found in Table 1.3 which shown accuracy result of dimethyl fumarate and Table 1.10 which shown accuracy result of cyclosporine and 1.12, respectively.

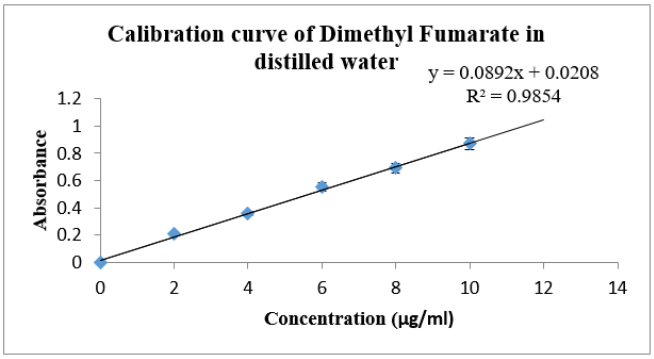


**Figure 1.7:** Calibration curve of Dimethyl Fumarate in distilled water

**Precision**

Precision studies were carried out to ascertain the reproducibility of the proposed method. Repeatability was determined by preparing six replicates of 6µg/mL concentration of the dimethyl fumarate and 2 µg/mL of cyclosporine and absorbance was measured using UV spectroscopy. Intraday precision study was carried out by preparing drug solution of same concentration and analyzing it at three different times in a day. The same procedure was followed for three different days to determine interday precision.

The result was reported as %RSD. The precision result of dimethyl fumarate and cyclosporine are shown in Table 1.4 and 1.11 respectively with percent relative standard deviation less than 2. The results of intraday precision study of dimethyl fumarate and cyclosporine are shown in Table 1.5 and 1.12, respectively**.**

The results of interday precision study of dimethyl fumarate and cyclosporine are shown in Table 1.6 and 1.13, respectively.

**Robustness**

The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in wavelength.

**Table 1.4:** Repeatability of Dimethyl fumarate

| *Replicates* | *Concentration (µg/ml)* | *Absorbance* |
|---|---|---|
| 1 | 6 | 0.542 |
| 2 | 6 | 0.548 |
| 3 | 6 | 0.540 |
| 4 | 6 | 0.539 |
| 5 | 6 | 0.541 |
| 6 | 6 | 0.545 |
| Mean | | 0.542 |
| Standard deviation | | 0.0033 |
| %RSD | | 0.625 |

| Drug | Change in Wavelength | | |
|---|---|---|---|
| Dimethyl fumarate | 208 nm | 210 nm | 212 nm |
| Cyclosporine | 212 nm | 214 nm | 216 nm |

The result of robustness study of the developed assay method of dimethyl fumarate is shown in Table 1.7 and Table 1.14 and cyclosporine is shown in 1.16.

**System suitability**

A system suitability test of the UV spectrophotometric system was performed before each validation run. Six replicate reading of standard preparation were taken and %RSD of standard reading were taken for same. System suitability of dimethyl fumarate is shown in Table 1.8 and Table 1.15 and cyclosporine is shown in Table 1.17.

## RESULTS AND DISCUSSIONS

**Analytical Method Development of Dimethyl Fumarate:**

Standard curves of dimethyl fumrate in distilled water and Phosphate buffer pH 5.5 were analyzed in the range of 2-10 µg/mL and 1-5µg/mL concentration respectively. The selected range of dimethyl fumarate was found to be linear.

The scan of drug solution in UV region (200-400nm) was done to find out the wavelength of maximum absorption (λmax). The λmax was found to be at 210nm. So the standard calibration curve of dimethylfumarate was developed at this wave length 210nm. The standard calibration curve of dimethylfumarate was obtained by plotting Absorbance vs. Concentration. Table 1.1 and 1.2, shows the absorbance values of dimethyl fumarate. The standard calibration curve is shown in Figure 1.1 and 1.2.

**Calibration curve of Dimethyl Fumarate in distilled water**

Distilled water is used because drug is freely soluble in distilled water.

**Calibration curve of Dimethyl Fumarate in Phosphate Buffer pH 5.5**

Phosphate buffer 5.5 is used because skin pH 5.5.

*Discussion*

Regression coefficients at 210 nm in distilled water and Phosphate buffer pH 5.5 were found to be 0.9854 and 0.9846, respectively.

Distilled water is used because drug is freely soluble in distilled water. Phosphate buffer 5.5 is used because skin pH 5.5.

The curve was found to be linear in the concentration range of 2-10 µg/mL for distilled water and 1-5 µg/mL for phosphate buffer 5.5 at 210 nm.

Regression co efficient for the drug in Distilled Water and PBS pH 5.5 were found to be near to one and in the linearity range. This standard concentration method obeys Beer's law and found to be suitable for the determination of drug entrapment and drug release study.

**Table 1.5:** Intraday precision for Dimethyl fumarate

| *Replicates* | *Concentration (µg/ml)* | *Absorbance* | | |
|---|---|---|---|---|
| | | *I* | *II* | *III* |
| 1 | 6 | 0.542 | 0.545 | 0.544 |
| 2 | 6 | 0.544 | 0.541 | 0.542 |
| 3 | 6 | 0.543 | 0.544 | 0.541 |
| 4 | 6 | 0.541 | 0.540 | 0.540 |
| 5 | 6 | 0.540 | 0.543 | 0.545 |
| 6 | 6 | 0.545 | 0.542 | 0.543 |
| Mean | | 0.543 | 0.543 | 0.543 |
| Standard deviation | | 0.002 | 0.002 | 0.002 |
| %RSD | | 0.345 | 0.345 | 0.345 |
| Average % RSD | | 0.345 | | |

**Table 1.6:** Interday precision for Dimethyl fumarate

| *Days* | *Concentration (µg/ml)* | *Absorbance* | | |
|---|---|---|---|---|
| | | *I* | *II* | *III* |
| 1 | 6 | 0.542 | 0.544 | 0.544 |
| 2 | 6 | 0.546 | 0.546 | 0.545 |
| 3 | 6 | 0.550 | 0.550 | 0.551 |
| Mean | | 0.543 | 0.546 | 0.550 |
| Standard deviation | | 0.001 | 0.001 | 0.001 |
| %RSD | | 0.213 | 0.106 | 0.105 |
| Average % RSD | | 0.141 | | |

**Table 1.7:** Change in wavelength for Dimethyl fumarate

| *Concentration of DMF* | *Change in Wavelength* | | |
|---|---|---|---|
| | *208 nm* | *210 nm* | *212 nm* |
| 6 | 0.521 | 0.545 | 0.532 |
| 6 | 0.522 | 0.545 | 0.532 |
| 6 | 0.522 | 0.544 | 0.534 |
| 6 | 0.523 | 0.543 | 0.534 |
| 6 | 0.521 | 0.544 | 0.535 |
| 6 | 0.521 | 0.545 | 0.535 |
| Mean | 0.522 | 0.544 | 0.534 |
| Standard deviation | 0.001 | 0.001 | 0.001 |
| %RSD | 0.157 | 0.150 | 0.256 |
| Average % RSD | 0.188 | | |

**Table 1.8:** System Suitability for Dimethyl fumarate

| *Concentration of DMF (µg/ml)* | *Absorbance* |
|---|---|
| 6 | 0.542 |
| 6 | 0.543 |
| 6 | 0.542 |
| 6 | 0.544 |
| 6 | 0.545 |
| 6 | 0.545 |
| Mean | 0.544 |
| Standard deviation | 0.001 |
| %RSD | 0.254 |

**Analytical method validation for Dimethyl fumarate[14,15,16]**

Proper wave length selection of the methods depends upon the nature of the sample and its solubility. To develop a rugged and suitable spectrophotometric method for the quantitative determination of dimethyl fumarate, the analytical condition were selected after testing the different parameters such as diluents, buffer, buffer concentration.

Our preliminary trials were by using different compositions of diluents consisting of water and phosphate buffer. Below figures represent the spectrums of blank, standard and test preparation respectively.

Scan standard solution in UV spectrophotometer between 200 nm to 400 nm on spectrum mode, using diluents as a blank.

*Selection of wavelength*

*Discussion:* The standard solutions containing dimethyl fumarate 1 µg/mL was scanned in UV range 200-400 nm using water as a blank. The wavelength corresponding to maximum absorbance in distilled water was found to be 210 nm.

*Accuracy*

Accuracy of the proposed method was determined using recovery studies. The recovery studies were carried out by adding different amounts (80%, 100%, 120%) of the pure drug to the pre-analysed formulation. The solutions were prepared in triplicates and the % recovery was calculated. The results of % recovery of dimethyl fumarate were shown in Table 1.3.

*Discussion:* Accuracy of the method is ascertained by standard addition method at 3 levels. Standard quantity equivalent to 80%, 100% and 120% is to be added in sample. The result shown that best recoveries **(99.55%-100.11%)** of the spiked drug were obtained at each added concentration, indicating that the method was accurate.

*Precision[17]*

Precision of the analytical method is ascertained by carrying out the analysis as per the procedure and as per normal weight taken for analysis. Repeat the analysis six times. Calculate the % assay, mean assay, % Deviation and % relative standard deviation and %RSD.

*Discussion:* The results of repeatability of DMF drug shown in Table 1.4 with %RSD. The results of intraday and interday precision studies are shown in Table 1.5, Table 1.6. The % RSD values for repeatability (0.625%), intraday (0.345%) and interday precision (0.141%) data were well below the specified limit of 2%. Hence, the method was specified in limit.

*Robustness*

The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in wavelength. If measurements are susceptible to variation in wavelength, the analytical condition should be suitably controlled or a precautionary statement should be included in the procedure.

*Discussion:* The result showed % RSD (0.188%) values to be within the acceptance criteria of 2%. Hence the method was found to be robust in variance wavelength.

*System Suitability*

A system suitability test of the spectrophotometric system was performed before each validation run. Six replicate reading of standard preparation were taken and %RSD of standard reading were taken for same. Acceptance criteria for system suitability, %RSD of standard reading not more than 2.0%, were full fill during all validation parameter

*Discussion:* Six replicate reading of standard preparation were taken and %RSD of standard reading were taken for same. Acceptance criteria for system suitability, %RSD of standard reading not more than 2.0%. Hence the method was validated.

**Table 1.9:** Calibration curve of Cyclosporine in Ethanol

| *SR No.* | *Concentration (µg/ml)* | *Mean Absorbance (±) SD* |
|---|---|---|
| 1 | 1 | 0.102±0.01 |
| 2 | 2 | 0.211±0.02 |
| 3 | 3 | 0.315±0.01 |
| 4 | 4 | 0.420±0.03 |
| 5 | 5 | 0.550±0.04 |
| 6 | 6 | 0.623±0.02 |
| 7 | 7 | 0.750±0.04 |
| 8 | 8 | 0.840±0.02 |

(Where n = 3, Mean± SD)

**Analytical Method Development of Cyclosporine[18,19]**

Standard curves of cyclosporine in ethanol were analyzed in the range of 1-8 µg/mL. The selected range of cyclosporine was found to be linear.

The scan of drug solution in UV region (200-400nm) was done to find out the wavelength of maximum absorption (λmax). The λmax was found to be at 214nm. So, the standard calibration curve of cyclosporine was developed at this wave length 214nm. The standard calibration curve of cyclosporine was obtained by plotting Absorbance vs. Concentration.

*Calibration curve of Cyclosporine in Ethanol*

Cyclosporine is freely soluble in ethanol.

*Discussion:* Standard curves of cyclosporine in ethanol were analyzed in the range of 1-8µg/mL concentration respectively. The selected range of cyclosporine was found to be linear. Regression coefficient at 214 nm in Ethanol was found to be 0.9984.

Regression co efficient for the drug in Ethanol was found to be near to one and in the linearity range. This standard concentration method obeys Beer's law and found to be suitable for the determination of drug entrapment and drug release study.

**Analytical Method Validation of Cyclosporine**

Proper wave length selection of the methods depends upon the nature of the sample and its solubility. To develop a rugged and suitable spectrophotometric method for the quantitative determination of dimethylfumarate, the analytical condition were selected after testing the different parameters such as diluents, buffer, buffer concentration.

**Table 1.10:** Accuracy of cyclosporine in ethanol

| *%Level* | *Replicates* | *Concentration of standard solution (µg/ml)* | *Concentration of sample solution (µg/ml)* | *Absorbance of Standard* | *Absorbance of Sample* | *% recovery* |
|---|---|---|---|---|---|---|
| | I | 10 | 10 | 0.551 | 0.554 | 99.46 |
| 80% | II | 10 | 10 | 0.552 | 0.553 | 99.82 |
| | III | 10 | 10 | 0.552 | 0.554 | 99.64 |
| | I | 10 | 10 | 0.662 | 0.663 | 99.85 |
| 100% | II | 10 | 10 | 0.661 | 0.663 | 99.70 |
| | III | 10 | 10 | 0.661 | 0.664 | 99.55 |
| | I | 1 | 1 | 0.335 | 0.338 | 99.11 |
| 120% | II | 1 | 1 | 0.334 | 0.338 | 98.81 |
| | III | 1 | 1 | 0.336 | 0.337 | 99.70 |

Our preliminary trials were by using different compositions of diluents consisting of water and phosphate buffer. Below figures represent the spectrums of blank, standard and test preparation respectively.

Scan standard solution in UV spectrophotometer between 200 nm to 400 nm on spectrum mode, using diluents as a blank.

*Selection of Wavelength*

*Discussion:* The standard Solutions containing cyclosporine 2 µg/mL was determine in UV range 200-400 nm using ethanol as a blank. The wavelength corresponding to maximum absorbance in ethanol was found to be 214 nm.

*Accuracy*[20]

Accuracy of the proposed method was determined using recovery studies. The recovery studies were carried out by

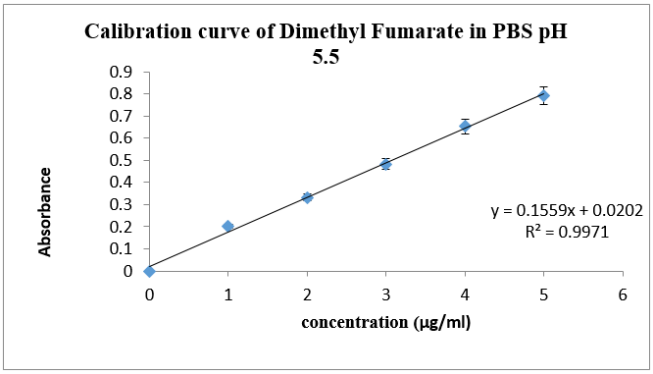


**Figure 1.8:** Calibration curve of Dimethyl Fumarate in PBS pH 5.5

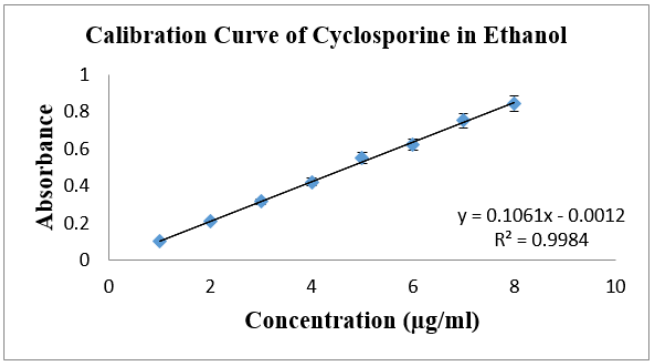


**Figure 1.9:** Calibration curve of Cyclosporine in Ethanol

**Table 1.11:** Repeatability of Cyclosporine in ethanol

| *Replicates* | *Concentration (µg/ml)* | *Absorbance* |
|---|---|---|
| 1 | 2 | 0.208 |
| 2 | 2 | 0.211 |
| 3 | 2 | 0.210 |
| 4 | 2 | 0.213 |
| 5 | 2 | 0.212 |
| 6 | 2 | 0.218 |
| Mean | | 0.212 |
| Standard deviation | | 0.003 |
| %RSD | | 1.607 |

**Table 1.12:** Intraday precision for cyclosporine in ethanol

| *Replicates* | *Concentration (µg/ml)* | *Absorbance* | | |
|---|---|---|---|---|
| | | *I* | *II* | *III* |
| 1 | 2 | 0.210 | 0.212 | 0.213 |
| 2 | 2 | 0.211 | 0.213 | 0.214 |
| 3 | 2 | 0.210 | 0.213 | 0.212 |
| 4 | 2 | 0.212 | 0.214 | 0.210 |
| 5 | 2 | 0.211 | 0.214 | 0.212 |
| 6 | 2 | 0.213 | 0.213 | 0.214 |
| Mean | | 0.211 | 0.213 | 0.213 |
| Standard deviation | | 0.001 | 0.001 | 0.002 |
| %RSD | | 0.554 | 0.353 | 0.714 |
| Average % RSD | | 0.540 | | |

**Table 1.13:** Interday precision for Cyclosporine in ethanol

| *Days* | *Concentration (µg/ml)* | *Absorbance* | | |
|---|---|---|---|---|
| | | *I* | *II* | *III* |
| 1 | 2 | 0.215 | 0.210 | 0.209 |
| 2 | 2 | 0.214 | 0.208 | 0.209 |
| 3 | 2 | 0.214 | 0.208 | 0.208 |
| Mean | | 0.214 | 0.209 | 0.209 |
| Standard deviation | | 0.001 | 0.001 | 0.001 |
| %RSD | | 0.269 | 0.553 | 0.277 |
| Average % RSD | | 0.366 | | |

adding different amounts (80%, 100%, 120%) of the pure drug to the pre-analysed formulation. The solutions were prepared in triplicates and the % recovery was calculated. The results of %recovery of cyclosporine were shown in Table 1.10.

*Discussion:* Accuracy of the proposed method was determined using recovery studies. The recovery studies were carried out by adding different amounts (80%,100%, and 120%) of the pure drug to the pre-analysed formulation. The solutions were prepared in triplicates and the % recovery was calculated. The results of Accuracy of cyclosporine in ethanol were shown in Table 1.11. Accuracy of the method is ascertained by standard addition method at 3 levels. Standard quantity equivalent to 80%, 100% and 120% is to be added in sample. The results show that best recoveries (99.11%-99.85%) of the spiked drug were obtained at each added concentration, indicating that the method was accurate.

**Table 1.14:** Change in wavelength for Cyclosporine

| *Concentration of Cyclosporine (µg/ml)* | *Change in Wavelength* | | |
|---|---|---|---|
| | *212 nm* | *214 nm* | *216 nm* |
| 2 | 0.198 | 0.210 | 0.245 |
| 2 | 0.198 | 0.211 | 0.246 |
| 2 | 0.201 | 0.210 | 0.247 |
| 2 | 0.202 | 0.209 | 0.247 |
| 2 | 0.202 | 0.211 | 0.246 |
| 2 | 0.202 | 0.212 | 0.246 |
| Mean | 0.201 | 0.211 | 0.246 |
| Standard deviation | 0.002 | 0.001 | 0.001 |
| %RSD | 0.985 | 0.498 | 0.306 |
| Average % RSD | 0.596 | | |

**Table 1.15:** System Suitability for wavelength for Cyclosporine

| *Concentration of Cyclosporine (µg/ml)* | *Absorbance* |
|---|---|
| 2 | 0.212 |
| 2 | 0.214 |
| 2 | 0.211 |
| 2 | 0.211 |
| 2 | 0.215 |
| 2 | 0.214 |
| Mean | 0.213 |
| Standard deviation | 0.002 |
| %RSD | 0.809 |

**Table 1.16:** Summary of Validation Parameter[23]

| *Method* | *Dimethyl fumarate* | *Cyclosporine* |
|---|---|---|
| $\lambda_{max}$ | 210 nm | 214 nm |
| Linearity | 2-10 µg/ml | 1-8 µg/ml |
| Regression Coefficient | 0.9854 | 0.9984 |
| Equation | Y=0.0892x +0.0208 | Y=0.0106x +0.0012 |
| Accuracy | 99.55%-100.11% | 99.11%-99.85% |
| Precision(Repeatability) | 0.625% | 1.607% |
| Intraday Precision | 0.345% | 0.540% |
| Interday Precision | 0.141% | 0.366% |
| Robustness | 0.188% | 0.596% |
| System Suitability | 0.254% | 0.809% |

*Precision*[21,22]

Precision of the analytical method is ascertained by carrying out the analysis as per the procedure and as per normal weight taken for analysis. Repeat the analysis six times. Calculate the % assay, mean assay, % Deviation and % relative standard deviation and %RSD.

*Discussion:* The results of repeatability of cyclosporine drug shown in Table 1.13 with %RSD. The results of intraday and interday precision studies are shown in Table 1.12, 1.13. The % RSD values for repeatability (1.607%), intraday (0.540%) and interday precision (0.366%) data were well below the specified limit of 2%. Hence, the method was specified in limit.

*Robustness*

The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in wavelength. If measurements are susceptible to variation in wavelength, the analytical condition should be suitably controlled or a precautionary statement should be included in the procedure.

*Discussion:* The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters.

If measurements are susceptible to variation in wavelength, the analytical condition should be suitably controlled or a precautionary statement should be included in the procedure.

The result showed % RSD values (0.596) to be within the acceptance criteria of 2%. Hence the method was found to be robust in variance wavelength.

*System Suitability*

A system suitability test of the spectrophotometric system was performed before each validation run. Six replicate reading of standard preparation were taken and %RSD of standard reading were taken for same. Acceptance criteria for system suitability, %RSD of standard reading not more than 2.0%, were full fill during all validation parameter

*Discussion:* Six replicate reading of standard preparation were taken and %RSD (0.809%) of standard reading were taken for same. Acceptance criteria for system suitability, %RSD of standard reading not more than 2.0%. Hence the method was validated.

## CONCLUSION

UV spectroscopy assay method development and validation of dimethyl fumarate and cyclosporine in nano dosage forms was successfully developed and based on results and discussion the developed methods were found to be simple, accurate, sensitive and reproducible. The developed methods can be successfully applied for estimation of dimethyl fumarate and cyclosporine in nano dosage form without any interference in quality control.

Analytical method validated by UV Spectrophotomer for Dimethyfumarate and cyclosporine at $\lambda_{max}$ 210 nm and 214 nm respectively. Summary of Validation Parameter is mentioned in below Table. 1.16.

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