# DEVELOPMENT AND VALIDATION OF ZERO ORDER UV SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF FORMOTEROL FUMARATE DIHYDRATE IN BULK AND IN PHARMACEUTICAL FORMULATION.

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## ABSTRACT

The current work is carried out for determination and validation of zero-order derivative spectroscopy of Formoterol Fumarate Dihydrate (FFD) in bulk and formulation by using UV-Spectrophotometer. For this purpose, the wavelength of 214 nm was selected. Sodium phosphate buffer was used as a solvent throughout the work. Linearity was observed in the concentration range of 2-12 µg/ml for the method. FFD obeyed Lambert Beer's law in the concentration range of 2 to 12 µg/mL ($r^2 = 0.9993$). The percent concentration in the Bulk drug was found to be 100.21 ± 0.03%, the percent concentration in marketed tablet Formulation was found 99.72 ± 0.02%. Parameters such as linearity, precision, accuracy, specificity, and ruggedness were studied as reported in the International Conference on Harmonization guidelines. The relative standard deviations for three replicate measurements in six concentrations of samples were found less than 2 %. The validation procedure confirms that this can be an appropriate method for their quantification in the formulation. The present method was found to be simple and linear which can be used for routine quality control analysis for spectrophotometric determination of zero-order derivative method in bulk or pharmaceutical formulation.

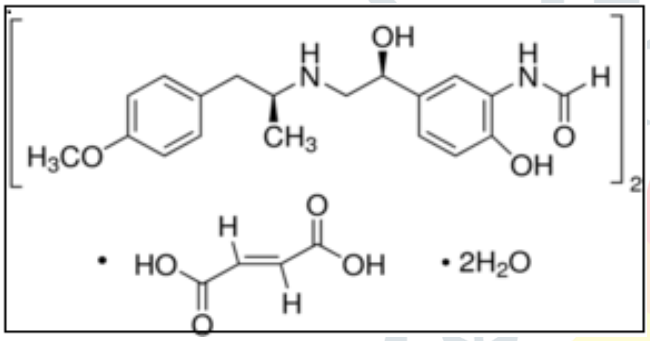
**Keywords:** Formetrol Fumarate Dihydrate (FFD), Antiasthmatic, Zero-Order derivative, λ max, UV.

## INTRODUTION

Formoterol, also known as eformoterol, is a long-acting β2 agonist used as a bronchodilator in the management of asthma and COPD. Formoterol has an extended duration of action compared to short-acting β2 agonists such as salbutamol which are effective for 4hr to 6 hr. LABAs such as formoterol are used as "symptoms controllers" to supplement prophylactic corticosteroid therapy. A reliever short-acting β2 agonist is still required since LABAs are not recommended for the treatment of acute asthma. Inhaled formoterol works like β2 agonists, causing bronchodilation by relaxing the smooth muscle in the airway to treat the exacerbation of asthma. [1-5]

Literature surveys revealed LC-MS [6] method for the quantitation of FFD in human urine, Assay of Formoterol by HPLC [7] and the spectrophotometric method by the formation of color chromogens of FFD has also studied for the estimation of FFD in pharmaceutical formulation. Many analytical methods such as RP-HPLC [8-11], UV- Spectrophotometry [12, 13] have been reported for the simultaneous determination of FFD in combination with Budesonide and Mometasone Furoate in Pharmaceutical formulation. To our knowledge, no simple and economic UV- Spectrophotometry methods were established in pieces of literature for the estimation of FFD in bulk and tablet dosage form. Therefore, the present work aims to establish a simple, accurate, precise, rapid and economic UV Spectrophotometric Zero order method for the determination of FFD in Pharmaceutical Bulk & dosage forms. Further to validate the developed analytical methods as per International Conference on Harmonization (ICH) guidelines. [14, 21]

**Figure 1. Chemical structure of Formoterol Fumarate Dihydrate.**




## MATERIAL AND METHOD:

### Instrumentation

A Shimadzu - 1800 UV-spectrophotometer Software with UV Probe 2.21 Matched quartz cells 1cm, Wavelength range 200-400 nm Lamp: 50 w, Deuterium Lamp Detector: Silicon Photodiode Cell holder: 1 mm wide, with above said parameters was used for the said research work.

### Solvent

Solubility of Formoterol Fumarate Dihydrate (FFD) was checked in different solvents and sodium phosphate buffer (pH 3) was selected as solvent

### Preparation of Buffer Solution

1.56 gm of $NaH_2PO_4.2H_2O$ was weighed and dissolved in 1000 mL double distilled water. The pH of this solution was adjusted to 3 by drop wise addition of sufficient amount of Ortho phosphoric acid. The buffer is then filtered through a 0.45µm membrane filter and sonicated.

**Preparation of stock standard solutions of Formetrol Fumarate Dihydrate**

10 mg of FFD was weighed and transferred to the 100 mL volumetric flask, to this 70 mL buffer solution was added, mixed well, sonicated, and the volume was made up to the mark by adding same solvent to obtain the stock solution of concentration 100µg/mL

**Determination of lambda maximum and selection of wavelengths**

The solution with concentration of 10µg/mL was prepared by diluting 1 mL of standard stock solution with 10 mL buffer solution. This solution was scanned in the UV-region (400 nm – 200 nm). In spectrum the FFD showed absorbance maximum at 214 nm as shown in fig 2.

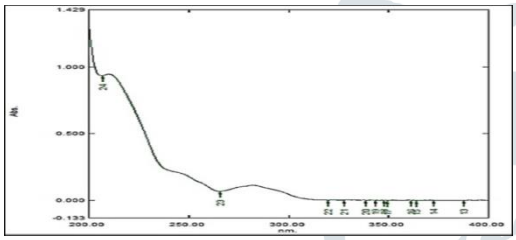
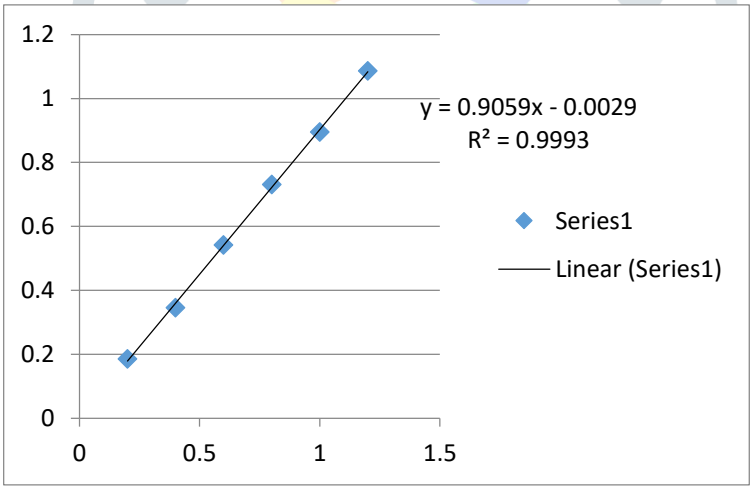


**Figure 2: The UV spectrum of Formoterol Fumarate Dihydrate at 214 nm**

**Figure 3: Calibration Curve of Formetrol Fumarate Dihydrate**




**Linearity study**

An appropriate volume of Formetrol Fumarate Dihydrate in the range of 0.2 – 1.2 mL were transferred into series of six separate 10 mL volumetric flasks and volume was made up to mark with buffer to obtain concentration of 2 – 12 µg/mL These solutions were scanned in UV-region 400 – 200 nm and absorbance was measured at 214 nm and a calibration curve was constructed as absorbance vs. concentration as shown in figure 3. It can be seen that the plot is linear over the concentration range of 2-12 µg/mL yielding a regression equation y = 0.0906x-0.0029 with a correlation coefficient $r^2$ = 0.9993. Absorbance is reported in Table 1 & Table 2.

**Table 1: Linearity Studies of Formoterol Fumarate Dihydrate**

| Concentration of FFD | Absorbance | % RSD |
|---|---|---|
| (µg/mL) | Mean ± SD (n = 6) | |
| 2 | 0.186 ± 0.029 | 1.391 |
| 4 | 0.346 ± 0.030 | 0.774 |
| 6 | 0.542 ± 0.042 | 0.694 |
| 8 | 0.731 ± 0.048 | 0.591 |
| 10 | 0.895 ± 0.049 | 0.494 |
| 12 | 1.087 ± 0.042 | 0.353 |

**Table 2: Linearity**

| Parameters | Formoterol Fumarate Dihydrate |
|---|---|
| Linearity range (µg/mL) | 02-12 |
| Slope | 0.0906 |
| Intercept | -0.0029 |
| Correlation coefficient ( $r^2$ ) | 0.9993 |

**Analysis of Bulk Material**

The aliquots of 6µg/mL solution were prepared from standard stock solution . The resulting solution was scanned at 214 nm. The concentrations of the drug were calculated from linear regression equations. The results are depicted in table 3. The % amount found was 100.21% with %RSD 0.55%. This indicates good recovery of drug. The analysis of capsule is reported Table 3.

**Table 3: Analysis of Bulk material (Formoterol Fumarate Dihydrate)**

| Amount taken (µg/mL) | Amount found (µg/mL) (n=6) | % Amount found Mean ± SD | %RSD |
|---|---|---|---|
| 6 | 6.01 | 100.21 ± 0.03 | 0.55 |

**Analysis of capsule formulation**:

100 Foradil capsules (Novartis) were taken and the contents were collected by removing the capsule shell and weighed. Then the contents equivalent to 1.2 mg was weighed accurately and transferred to the 10 mL volumetric flask. To this 7 mL buffer solution was added, mixed and sonicated. Then the volume was made up to the mark by addition of same solvent to obtain desired conc. 120µg/mL and it was filtered through 0.45µm

membrane filter. From this solution 0.5mL was taken and diluted up to 10mL with same solvent. The resulting solution was scanned at 214 nm. The concentrations of the drug were calculated from linear regression equations. The results are depicted in table… The % amount found was 99.72% with %RSD 0.39%. This indicates that no interference of excipients from capsule formulation. The analysis of capsule is reported Table 4.

**Table 4: Analysis of Capsule formulation (Foradil Capsule)**

| **Amount taken (µg/mL)** | **Amount found (µg/mL) (n=6)** | **% Amount found Mean ± SD** | **%RSD** |
|---|---|---|---|
| 6 | 5.98 | 99.72 ± 0.02 | 0.39 |

**Validation of method**

The proposed method is validated as per ICH guidelines for various parameters like accuracy, precision, ruggedness and sensitivity.

**Accuracy**

To the pre-analyzed sample solutions, a known amount of standard stock solution was added at different levels i.e. 80 %, 100% and 120%. The solutions were reanalyzed by proposed method; results of recovery studies are reported in Table 5. Results have shown that the recovery of Formetrol Fumarate Dihydrate is within 90.79-99.45% with %RSD less than 2% is reported in Table 5.

**Table 5: Accuracy**

| Initial amount | Amount of standard drug added | Amount of standard drug added | Amount recovered | % Drug recovered | % RSD |
|---|---|---|---|---|---|
| (µg/mL) | (µg/mL) | (%) | (µg/mL) | Mean ± SD | |
| 6 | 4.8 | 80 | 10.74 | 98.79 ± 0.01 | 0.16 |
| 6 | 6 | 100 | 11.97 | 99.45 ± 0.02 | 0.23 |
| 6 | 7.2 | 120 | 13.13 | 99.03 ± 0.02 | 0.17 |

| Initial amount (µg/mL) | Amount of standard drug added (µg/mL) | Amount of standard drug added (%) | Amount recovered (µg/mL) | % Drug recovered Mean ± SD | % RSD |
|---|---|---|---|---|---|
| 6 | 4.8 | 80 | 10.74 | 98.79 ± 0.01 | 0.16 |
| 6 | 6.0 | 100 | 11.97 | 99.45 ± 0.02 | 0.23 |
| 6 | 7.2 | 120 | 13.13 | 99.03 ± 0.02 | 0.17 |

**Precision**

Precision of the method was studied as repeatability, intra-day and inter-day precision. these parameters were determined by estimating the corresponding response 3 times on the same day and on 3 different days over period of one week for 3 different concentrations of formoterol fumarate dehydrate (6µg/mL) for six times and the results of precision and repeatability are reported in Table 6.

**Table 6: Precision**

| Precision | Amount taken (µg/mL) | Amount found (µg/mL) | % Amount found (µg/mL) | % RSD |
|---|---|---|---|---|
| **Repeatability (n=6)** | 6 | 5.99 | 99.78 ± 0.02 | 0.49 |
| **Intra-day (n=3)** | 4 | 3.95 | 98.91 ± 0.02 | 0.67 |
| | 6 | 5.96 | 99.40 ± 0.02 | 0.47 |
| | 8 | 7.97 | 99.67 ± 0.02 | 0.35 |
| **Inter-day (n=3)** | 4 | 3.97 | 99.34 ± 0.02 | 0.67 |
| | 6 | 5.95 | 99.27 ± 0.02 | 0.44 |
| | 8 | 7.95 | 99.36 ± 0.01 | 0.22 |

**Ruggedness**

Ruggedness of the proposed method was determined by analyzing aliquots from homogenous slot (6 µg/mL) in different analysts using similar operational and environmental conditions. The results showed that there is almost recovery of drug and percent relative standard deviation is less than 2% as shown in Table 7.

**Table 7: Ruggedness**

| Amount taken 6 (µg/mL) | Analyst –I | | | Analyst –II | | |
|---|---|---|---|---|---|---|
| | Amount found (µg/mL) | % Amount found Mean ± SD (n = 6) | % RSD | Amount found (µg/mL) | % Amount found Mean ± SD (n = 6) | % RSD |
| | 5.98 | 99.69 ± 0.03 | 0.50 | 5.99 | 99.81 ± 0.02 | 0.48 |

**Sensitivity**

Sensitivity of the proposed method was estimated in terms of limit of quantitation (LOQ) and limit of detection (LOD). The LOD and LOQ were calculated by the use of equation LOD = 3.3 * ASD/S and LOQ=10 * ASD/S; where, `ASD´ is Average standard deviation of the peak areas of the drug (n= 3). Taken as a measure of noise and "S" is the slope of the corresponding caliberation curve. The procedure was repeated in triplicate. The results are shown in Table 8**.** LOD and LOQ values found to be 0.11µg/mL, respectively.

**Table 8: Sensitivity**

| | |
|---|---|
| LOD(µg/mL) | 0.11 |
| LOQ(µg/mL) | 0.32 |

**CONCLUSION**

This attempt to develop a zero order UV spectrophotometric derivative technique is quite simple, accurate, precise, reproducible and sensitive for quantification of Formetrol Fumarate Dihydrate in capsule formulation. FFD obeyed Lambert Beer's law in the concentration range of 2 to 12 µg/mL ($r^2$ = 0.9993). The validation procedure confirms that this can be an appropriate method for their quantification in the formulation. It can also be used in routine quality control of the raw materials as well as formulation containing this entire compound.

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**REFERENCES**


1. Skoog D A, West DM. Principles of Instrumental Analysis: 2nd edition, United States of America: Saunders golden sunburst series; 1980. Page 148-188, 666-709
2. Khopkar S. M. Basic concepts of Analytical Chemistry: 3rd ed. New age international; 2008. 1-2,113-115.
3. Christian G. D. Analytical Chemistry: 6th ed. Singapore: John Wiley and sons (Asia) pvt. Ltd; 2007. Page 1-5.
4. Somenath M. Sample Preparation Techniques in Analytical Chemistry: 1st ed. New York: Wiley Interscience; 2003. Page 13-16,105.
5. Sharma BK. Instrumental Methods of Chemical Analysis: 11th ed. Meerut: Goel Publishing House; 1991. Page 1-9.
6. Youxuan Xu. Genye He, Jianghai Lu, Xiaobing Wang, Yun Wu, et.al. (2013) An Improved Liquid Chromatography– Tandem Mass Spectrometric Method to Quantify Formoterol in Human Urine. Journal of Chromatographic Science Advance 12: 1-4.
7. Appala Raju (2010) A High Performance Liquid Chomatographic Assay for Formoterol in Bulk Drug and its Pharmaceuti cal Dosage Forms ijbar 2: 118-24.
8. Pai N, Patil SS (2013) Development and validation of RP-HPLC method for estimation of Formoterol fumarate and budesonide in pressurised meter dose inhaler form. Der Pharmacia Sinica 4: 15-25.
9. Ahmed S, Jayakar B, Aleem MA (2011) Development of Reverse Phase High Performance Liquid Chromatography Method and its Validation for Estimation of Formoterol Fumarate Rota Caps. IJPSR, 2: 319-24.
10. Srinivasarao K, Gorule V, Venkata RC, Venkata KA (2012) Validated Method Development for Estimation of Formoterol Fumarate and Mometasone Furoate in Metered Dose Inhalation Form by High Performance Liquid Chromatography. J Anal Bioanal Techniques 3: 1-4.
11. Kale NR, Pingle AP, Mirza JA, Dhongade GN (2014) Development and Validation of Stability-Indicating RP-HPLC Method for Simultaneous Estimation of Formoterol Fumarate and Budesonide in Metered Dose Inhaler Formulation WJPR 3: 1386-99.
12. Gurjar, Neema M, Seth AK, Zanwar Aarti, et al. (2012) Development of First Derivative Spectroscopy Method for Estimation of Budesonide and Formeterol in Combined Dosage Form. Int J Pharm sci 3: 82-92.
13. Prasad AVSS (2006) Simultaneous Spectrophotometric Determination of Formoterol Fumarate and Budesonide in their Combined Dosage Form. Indian Journal of Chemical Technology 13: 81-83.
14. Beckett AH., Stenlake JB., Practical Pharmaceutical Chemistry: 4th ed. Part II, New Delhi: CBS Publications and Distributors; 1997. Page 1, 275-300.
15. Gary C. D. Analytical chemistry: 5th ed. John Wiely & sons; 2001, Page 1- 3.

16. Gennaro AR. Remington: The Science and Practice of Practice of Pharmacy: 21st ed. volume II Lippincott; Williams and welkins Mack Publishing Company; Page 1680-82.
17. Munson JW. Pharmaceutical Analysis modern methods: 1st ed. Marcel Dekker series of books; 2001. Page 30-50.
18. Willard M., Dean S. Instrumental method of analysis: 7th edition. New Delhi: CBS publication and distributors; 1986. Page 3-5
19. ICH, Q 2(R1), Harmonized Tripartite Guideline, Test on Validation of Analytical Procedures: Text and methodology, in: Proceedings of the International Conference on Harmonization, Geneva, October, 1994.
20. ICH, Q2B, Guideline for Pharmaceutical Industry, Text on Validation of Analytical Procedures: Methodology, Geneva, November 1996.
21. ICH, Q2A, Guideline for Pharmaceutical Industry, Text on Validation of Analytical Procedures, Geneva, March 1995.
22. Pharmacopoeia I. volume II. Government of India Ministry of Health & Family Welfare: The Indian Pharmacopoeia Commission. Ghaziabad, India. 2014: Page 934, 1824.
23. Pharmacopoeia B. volume II. British Pharmacopoeial Commission, London, United Kingdom. 2005; Page 882.
24. Jain HK, Bhangale P R. Spectrometric method for simultaneous estimation of Formoterol Fumarate and Mometasone Furoate in Resicaps. International Research Journal of Pharmacy. 2013 4(6):220-223