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Validated UV Spectrophotometric area under curve Method for Determination of Formoterol Fumarate Dihydrate in Bulk and Pharmaceutical Formulation using Hydrotropic Solubilization Technique

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Abstract

A novel, safe, accurate, sensitive and economic two UV-Spectrophotometric methods "Method A" zero order and "Method B" Area under curve (AUC) was developed by the application of hydrotropic solubilization technique using 1% ammonium acetate solution as hydrotropic solubilizing agent for the quantitative determination of poorly water-soluble Formoterol Fumarate Dihydrate (FFD) from bulk and tablet dosage form. The solubility of FFD increases hydrotropic solution as compared to solubility in distilled water, thus, 1% ammonium acetate hydrotropic solution was employed to carry out UV-spectrophotometric analysis precluding use of organic solvents. FFD obeyed Lambert Beer's law in the concentration range of 10 to 60µg/mL (r2=0.999) for both methods in hydrotropic agent with mean recovery was found 99.23 ± 0.83% (Method A) and 99.42 ± 0.42 for (Method B). The percent concentration in marketed tablet formulation was found 99.60 ± 0.78% (Method A) and 99.69 ± 1.07% (Method B). 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 always less than 2%. So this method can successfully employ in the routine analysis of FFD in bulk drug and pharmaceutical formulation.

Keywords: Formoterol fumarate dihydrate; FFD; UV-spectrophotometry; Area under curve technique

Introduction

Increasing the aqueous solubility of insoluble and slightly soluble drugs is of major importance. Various techniques have been employed to enhance the aqueous solubility of poorly water-soluble drugs. Hydrotropic solubilization is one of them [1]. The term hydrotropy has been used to designate the increase in solubility of various poorly water-soluble compounds due to the presence of a large amount of water soluble additives [2].

Sodium benzoate, niacinamide, sodium salicylate, sodium acetate, sodium citrate, and urea, have been employed to enhance the aqueous solubility of many poorly water soluble drugs. Various organic solvents like methanol, chloroform, alcohol, dimethyl formamide, and benzene have been employed for the solubilization of poorly water soluble drugs for spectrophotometric estimation [3].

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Drawbacks of organic solvents include higher cost, toxicity, pollution, and error, in analysis due to volatility. The primary objective of this study was to employ hydrotropic solubilizing agents, sodium citrate and urea to preclude the use of organic solvents.

Formoterol Fumarate Dihydrate (FFD), N [2 hydroxy 5 (1 hydroxy 2 {[1 (4 methoxyphenyl) propan 2 yl]amino}ethyl)phenyl]formamide (Figure 1) is a long-acting β2-selective adrenoceptor agonist, used as Bronchodilator and also used in Chronic Obstructive Pulmonary Disease (COPD). It acts as stimulator for the β2 adrenergic receptors in bronchial smooth muscle [4,5].

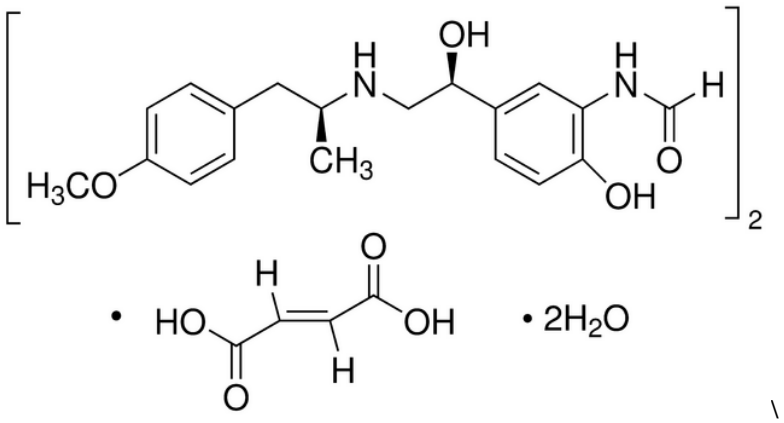

Figure 1: Structure of FFD (Formoterol Fumarate Dihydrate).

Literature survey revealed LC-MS [6] method for quantitation of FFD in human urine, Assay of Formoterol by HPLC [7] and spectrophotometric method by formation of colour 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 simultaneous determination of FFD in combination with Budossunide and Memontasone Furoate in Pharmaceutical formulation.

To our knowledge, no simple and economic UV- Spectrophotometry methods were established in literature for the estimation of FFD in bulk and in tablet dosage form.

Therefore, the aim of the present work is to establish simple, accurate, precise, rapid and economic UV – Spectrophotometric (Zero order and AUC) methods for determination of FFD in Pharmaceutical dosage forms. Further to validate the developed analytical methods as per International conference on Harmonization (ICH) guidelines.

Selection of solvent

Solubility of Formoterol Fumarate Dihydrate (FFD) was checked in different solvents according to literature survey and the solubility were improved in water by using 1% Ammonium acetate solution was selected as solvent.

Preparation of stock standard solutions of FFD

Stock standard solution of FFD was prepared by dissolving 10mg drug into 100mL of 1% Ammonium acetate solution to obtain concentration 100 µg/mL.

Linearity study

From the stock standard solution, an appropriate volume in the range of 1-6 mL were transferred into a series of 10 mL volumetric and volume was made up to mark using 1% Ammonium acetate solution to obtain concentrations in the range of 10-60 µg/mL. The solutions were scanned on a spectrophotometer in the range of 400–200 nm. For "Method A" absorbance in zero order spectrum was determined at 281 nm (**Figure 2)** and for "Method B" AUC in zero order spectrum was selected in range of 265.20- 295.20 nm (**Figure 3**).

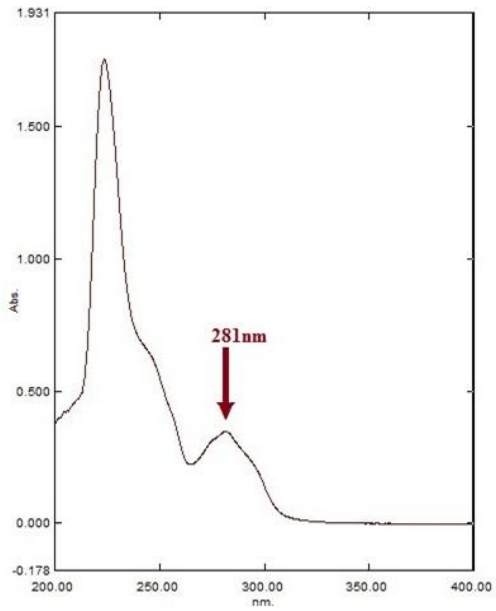

Figure 2: UV Spectrum of FFD showing maximum absorbance at 281.00 nm.

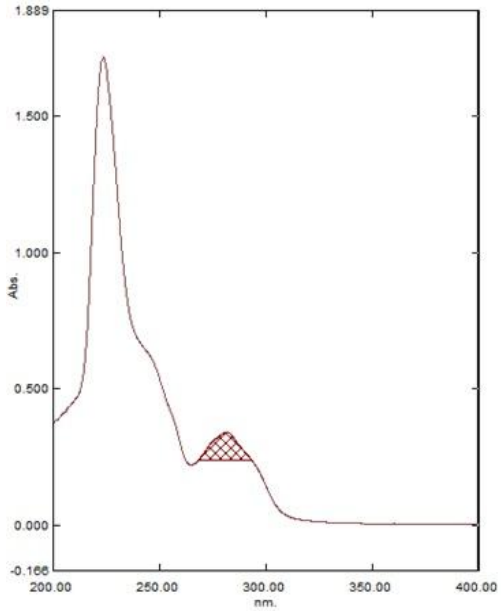

Figure 3: UV Spectrum of FFD showing area at wavelength range 265.20-295.20 nm.

Analysis of bulk material

Accurately weighed quantities 10 mg FFD were taken in 100 mL of volumetric flask and dissolved in 50mL 1% ammonium acetate solution by vigorous shaking. The volume was made up to the mark by same solvent. The aliquot portions of this stock solution were further diluted with solvent to get final concentration of about 40µg/mL FFD and the AUC was measured at selected wavelengths and results are reported in Table 1.

Method	**Amount Taken (µg/mL)**	**%Amount found**	**%RSD (n=6)**
A	40	99.94	0.65
B	40	99.95	1.04

Table 1: Analysis bulk Material

Analysis of marketed formulation

Average weight of twenty capsules were taken, and capsules shell was separated transferred to a clean dry mortar, and grounded into a fine powder using a pestle .An amount of powder equivalent to 10mg FFD was transferred into 100 mL

volumetric flask containing 35 mL of 1% Ammonium acetate solution and, sonicated for 20 min, and solution was diluted to volume with same solvent. The resulting solution was filtered through Whatmann filter paper no. 41. An appropriate volume of sample was taken and diluted with water to get the final concentration of 4.0 µg/mL. The responses measured and concentrations in the sample were determined from respective linearity equations Table 2.

Method	**Amount Taken (µg/mL)**	**%Amount found**	**%RSD (n=6)**
A	40	99.60	0.78
B	40	99.69	1.07

Table 2: Analysis of marketed Formulation**.**

Validation of the proposed methods

The proposed methods are validated according to International Conference on Harmonization (ICH) guidelines.

Accuracy

To the pre analyzed sample solutions, a known amount of drug standard solution was added different levels i.e. 80%, 100% and 120%. The solutions then were re-analyzed by proposed method [13-15]. For both methods (Method A and B) to the pre-analyzed sample solution, a known amount of drug standard was added and re-analzyed by proposed respective UV Spectrometry analytical methods in Table 3.

Methods	**% level**	**% Recovery [n=3]**	**% RSD [n=3]**
A	0	100.09	0.43
	80	99.05	0.87
	100	99.24	0.69
	120	99.41	0.94
B	0	100.12	0.58
	80	98.87	0.37
	100	99.54	0.41
	120	99.85	0.47

n- number of determinations at each level

Table 3: Accuracy Studies.

Repeatability

Repeatability was determined by analyzing FFD (40µg/mL) for six times and the results are reported in Table 4.

Method	**Amount Taken (µg/mL)**	**Amount Found**	**+ SD**	**% RSD**
A	40	40.11	0.30	0.74
B	40	40.24	0.14	0.35

Table 4: Repeatability.

Precision

The intra-day and inter-day precisions of the UV- Spectrophotometry analyses were evaluated using linear regression data for the calibration curves; analyzing FFD repeatedly at concentrations of 30, 40 and 50 µg/mL for UV- Spectrophotometry determinations (Table 5).

Methods	Concentrations (µg/mL)	Intra-day (% RSD) [n=3]	Inter-day (% RSD) [n=3]
A	30	1.85	0.71
	40	0.74	0.84
	50	0.59	1.47
B	30	0.31	1.00
	40	0.69	0.77
	50	0.41	0.45

Table 5: Precision Studies*.

Ruggedness

Ruggedness of the proposed method is determined by analysis of aliquots from homogenous slot by two analyst using same operational and environmental conditions.

Sensitivity

Sensitivity of the proposed method was estimated in terms of limit of detection (LOD) and limit of quantitation (LOQ). The LOD and LOQ were calculated by the use of the equation LOD=3.3 * ASD/SD and LOQ=10 * ASD/SD; where, 'ASD' is Average standard deviation of the peak area areas of the drug (n=3), taken as a measure of noise, and 'SD' is the slope of the corresponding calibration curve. The procedure was repeated in triplicate and the results are reported in Table 6.

Parameters	Method A	Method B
Linearity range (µg/mL)	10-60	10-60
Slope	0.011	0.060
Intercept	0.002	0.014
Correlation coefficient(r2)	0.999	0.999
LOD (µg)	1.38	1.28
LOQ (µg)	3.17	3.87

Table 6: Optical characteristics and linearity data of FFD.

Conclusion

An attempt was made to develop a novel, safe, accurate, sensitive and economic UV-Spectrophotometric methods by the application of hydrotropic solubilization technique using 1% ammonium acetate solution as hydrotropic solubilizing agent for the quantitative determination of poorly water-soluble Formoterol Fumarate Dihydrate (FFD) from bulk and tablet dosage form. FFD obeyed Lambert Beer's law in the concentration range of 10 to 60 µg/mL (r2=0.999) in hydrotropic agent with mean recovery was found 99.23 ± 0.83% (Method A) and 99.42 ± 0.42 for (Method B). The percent concentration in marketed tablet formulation.

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