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A Review On Analytical Methods For Estimation of Formoterol Fumarate In Pharmaceutical Dosage Form and Biological Fluids

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ABSTRACT

Formoterol fumarate is a selective long acting β_2 adrenergic receptor agonist indicated for the treatment of chronic obstructive pulmonary disease (COPD). Various analytical methods used for the estimation of Formoterol fumarate alone and combination with other drugs have been reviewed in this paper. These are include Ultraviolet spectrometry, High performance thin layer chromatography, High performance liquid chromatography, Ion pair to determine the amount of Formoterol fumarate in bulk drugs and pharmaceutical formulations as well as in Biological Fluids. Stability indicating and impurity profiling methods for Formoterol fumarate are also described. These analytical methods can be used for qualitative and quantitative estimation of Formoterol fumarate and / or its related impurities or degradants in bulk and formulation.

Keywords: Formoterol fumarate, analytical methods, estimation, formulation and Biological Fluids.

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INTRODUCTION

Formoterol Fumarate dehydrate (FF) is chemically known as N-[2-hydroxy-5-[(1RS)-1hydroxy-2- [[(1RS)-2- (4-methoxy-phenyl) –methyl ethyl] amino] ethyl] phenyl] formamide(E)-butanedioate dehydrate (Figure 1).This drug is official in USP [1], EP [2] , IP [3] and BP [4].

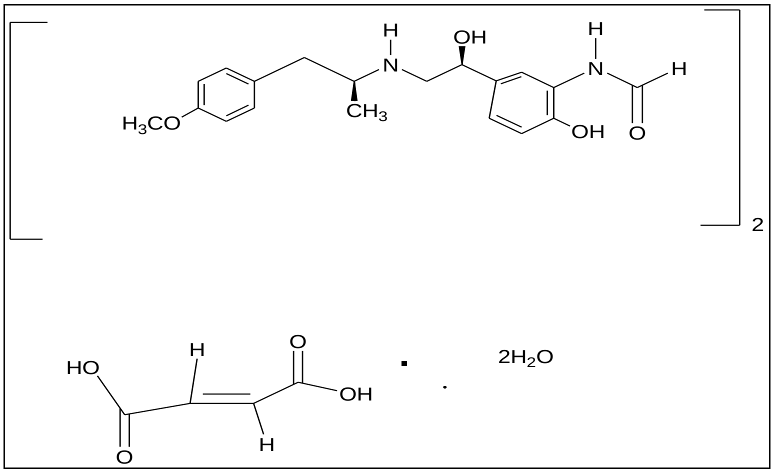

Figure 1: Chemical structure of Formoterol fumarate

It is a potent selective long acting β_2 adrenergic receptor agonist which acts as bronchodilator for the treatment of asthma by relaxing muscle spasms in the air passages of lungs upon inhalation. It stimulate intracellular adenyl cyclise that catalyse conversion of adenosine triphosphate (ATP) to cyclic 3’, 5’-adenosine monophosphate (cAMP). Increased cAMP levels cause relaxation of bronchial smooth muscle and inhibition of mediators of immediate hypersensitivity from cells, especially from mast cells such as histamine and leukotrienes, from the human lung.

90% of Formoterol fumarate administered from an inhaler will be swallowed and then absorbed from the gastrointestinal tract. Peak plasma concentrations of the unchanged substance are reached 0.5 to 1 hour after administration.

The plasma protein binding of Formoterol is 61 to 64% (34% to albumin). There is no saturation of binding sites in the concentration range reached with therapeutic doses. It metabolized in the liver by CYP isoenzymes 2D6, 2C19, 2C9, and 2A6.

Formoterol fumarate is eliminated primarily by metabolism, direct fold glucuronidation being the major pathway of biotransformation. O-demethylation followed by glucuronidation is another pathway.

The drug and its metabolites are completely eliminated from the body; about two-thirds of an oral dose appears in the urine and one-third in the feces. After inhalation about 6 to 9% of the dose on average, it is excreted unchanged in the urine. Renal clearance of Formoterol is 150mL/min.

Side effects of Formoterol fumarate, Patient with asthma are viral infection, bronchitis, chest infection, dyspnea, chest pain, tremor, dizziness, insomnia, tonsillitis, rash, dysphonia, and serious asthma exacerbation (dose related).

Side effects of Formoterol fumarate Patient with COPD are Upper respiratory tract infection, back pain, pharyngitis, chest pain, sinusitis, fever, leg cramps, muscle cramps, anxiety, pruritus, increased sputum (dose related) and dry mouth [5].

Analytical Methods for Estimation of Formoterol Fumarate in Bulk Drug, Pharmaceutical Formulation and Biological Fluids

Many different analytical methods have been reported for the estimation of Formoterol Fumarate (FF) in bulk and dosage form as well as in biological fluids.

Spectrophotometric methods:

Gousuddin M et al. developed and validated three simple, sensitive, selective, accurate, precise and economical methods (method A, B and C) for the quantitative estimation of Formetrol in bulk drug and its pharmaceutical formulations. Methods were based on the formation of coloured chromogens using 2,2'- Bipyridyl in presence of Ferric chloride in method A, Potassium Fericicynide in presence of $FeCl_3$ in method B and Gibb's reagent in presence Borax in method C which were orange ,dark green, and dark blue in colour respectively. Absorptions were measured at 520 nm, 756 nm, and 618 nm respectively for all three colored solutions. Linearity range was found to be 2-10 µg/ml in all the three methods. The result was found to be accurate, and free from the interference of rotacaps excipients [6].

Aarti. S. Zanwar et al. developed two spectrophotometric methods, First derivative zero crossing spectrophotometry and ratio- spectra derivative spectrophotometric methods for the determination of Mometasone furoate (MF) and Formoterol fumarate (FF) in the rotacaps. First method involves amplitudes measurement of the first derivative spectra of the standard and sample solution at 267.56 nm for MF and 306 nm for FF. Second method involves amplitudes measurement of the ratio first derivative spectra at 263nm and 267.30 nm for MF and 293.08 and 302.60 nm for FF. Linearity of responses achieved in the range of 30-200 µg /ml of MF and 2-6 µg /ml for FF. The methods validated statistically for the accuracy and precision. These developed methods preferable for routine analysis due to its simplicity and economical advantages [7].

Bhangale PR et al. developed and validated a UV-Spectrophotometric method for the simultaneous estimation of Formoterol Fumarate (FF) and Mometasone Furoate (MF) in respicaps dosage form. This method is based on area under curve (AUC) in the wavelength range of 210-220 nm and 242-252 nm. Linearity was found in the concentration ranges of 3-18 µg/ml and 6-21 µg/ml for FF and MF, respectively. % Assay of marketed formulation was found to be 98.18 ± 0.105 98.33 ± 0.884 for FF and MF respectively suggest that no interference of excipient. LOD was found to be 0.0491 µg /ml and 0.278 µg/ml for FF and MF respectively. LOQ was found to be 0.016 µg/ml and 0.0195 µg /ml respectively for FF and MF. This method was validated as per International Conference on Harmonization (ICH) guidelines. Validated method can be satisfactorily applied for routine analysis of pharmaceutical dosage form [8].

Prasad AVVS developed a new UV spectrophotometric method for the simultaneous determination of Formoterol fumarate (FF) and Budesonide (BD) in their combined dosage form. For the formation of simultaneous equation, the λ_{max} values for FF and BD in methanol medium were found to be 217 nm and 252 nm respectively. Method was found to be linear in the range of 1.5-7.5 µg/ml for FF and 25-125 µg/ml for BD. This method was found to be precise and accurate on the basis of achieved results of the analysis [9].

Gurjar NM et al. developed sensitive, precise, accurate and simple first order derivative spectrophotometric method for simultaneous estimation of Budesonide (BD) and Formoterol (FRL) in dry powder inhaler dosage form. First order derivative amplitudes were measured at selected wavelengths, 269.4 nm for BUD and 284.6 nm for FRL. Zero crossing point was found to be 269.4 nm and 284.6 nm for FRL and BUD respectively. Linearity was found to be in the concentration range of 2-26 µg/ml for BUD and 10-100 µg /ml for FRL. The mean % recovery of the method was achieved 100.07% for BUD and 100.06% for FF. The method was precise with %RSD 0.41 and 0.96 for BUD and FRL (API) respectively (for intraday) and % RSD 0.42 and 0.84 for BUD and FRL (combined dosage form) respectively (for intraday) and 0.78 and 0.48 for BUD and FRL (API) respectively (for inter-day) and 0.61 and 0.45 for BUD and FRL (combined dosage form) respectively (for interday). The proposed methods have been applied successfully to the analysis of cited drugs in pharmaceutical formulations. The method was validated as per ICH guidelines [10].

Duygu T et al. developed rapid, simple, accurate and sensitive spectrophotometric method for the determination of Formoterol fumarate dihydrate in bulk and dosage forms. The method is based on the formation of yellow coloured ion pair complex due to the reaction of Formoterol fumarate dihydrate (FF) and methyl orange (MO) at pH 4 in chloroform and measurement of absorbance of colored complex. Ion pair complex has a λ_{max} at 428 nm after extraction with chloroform and a linear calibration over the range of 4-20 µg/mL. Limit of detection was 0.22 µg/mL and limit of quantification was 0.66 µg/mL. The proposed method has been applied to the assay of FF commercially available capsules. The method was found to be accurate and reproducible. No interference was observed from common excipients of tablets [11].

Shah PD et al. developed two simple, rapid and precise UV spectroscopic methods, Simultaneous equation (method 1) and Q-absorbance ratio (method 2) for the simultaneous determination of Beclomethasone dipropionate (BD) and Formoterol fumarate (FF). In method 1, linearity was found over the concentration range of 10-50 μg/ml for BD and 1-5 μg/ml for FF at 239.2 nm (R^2=0.998 and 0.998 respectively) and 213nm (R^2=0.999 and 0.998 respectively) wavelengths respectively. Method 2 involves the formation of Q absorbance equation using the absorptivity values at 218.3 nm (isoabsorptive point) (R^2=0.998 and 0.998 respectively) and 239.2nm (λ_{max} of BD) (R^2=0.998 and 0.999 respectively). Linearity was found in concentration range of 10-50 μg/ml and 1-5 μg/ml for respectively. The proposed methods were validated according to ICH guidelines for evaluation of accuracy, precision, sensitivity etc. The proposed methods was found to be novel, simple, accurate, precise, sensitive, rapid and economically viable methods that do not require any prior separation procedure. The proposed two methods hold potential for simultaneous determination of BD and FF in rotacap formulation [12].

Chromatographic methods:

The high performance thin layer chromatography (HPTLC) and high performance liquid chromatography (HPLC) methods have been reported for the analysis of Formoterol fumarate in pharmaceutical formulation as well as in bulk.

HPTLC (High Performance Thin Layer Chromatography)

Patil AS developed a novel, simple, rapid and reliable High-Performance Thin-Layer Chromatographic (HPTLC) method and validated for the analysis of Formoterol Fumarate Dihydrate (FFD) in bulk and in in-house capsules formulation. FFD was quantify by UV detection at 281 nm and analysis was performed on (10 cm × 10 cm) aluminium sheets precoated with silica gel 60-F254 (E. Merck) as stationary phase and ethyl acetate: methanol: triethylamine (3.2:1.5:0.3 *v/v*) as mobile phase. Quantitation by HPTLC method was carried out over the concentration range of 400 - 900 ng /band. The HPTLC method resulted into compact and well resolved band for FFD at retention factor (R_f) of 0.51 ± 0.3. Linearity for calibration of HPTLC method has showed good linear relationship with regression coefficient; R^2 = 0.999. The developed method was validated as per ICH guidelines. Degradation study was carried out by acid and alkali hydrolysis, oxidation, neutral, photo and thermal degradation of FFD. The drug was degrading under acidic, basic, oxidation, and thermal conditions. This indicates that the drug is susceptible to acid, base, oxidation and thermal conditions. The degraded product was well resolved from the pure drug with significantly different R_f value. Statistical analysis proved that the developed methods were precise, robust, sensitive and accurate and can be used effectively for the analysis of FFD in bulk and pharmaceutical formulations [13].

Merey HA developed chromatographic method and validated for the simultaneous determination of Mometasone furoate (MO) and Formoterol fumarate dihydrate (FOR). Combination of MO and FOR is used for the treatment of asthma in patients suffering from reversible obstructive airway disease. The method was performed using aluminum TLC plates pre-coated with silica gel GF_{254} as the stationary phase and chloroform:ethyl acetate: methanol:toluene:formic acid (5:2:2:2:0.1, by volume) as the mobile phase followed by densitometric measurement of the separated bands at 233 nm. Linearity was found within concentration range 2-14 µg/band for MO and 0.1-5 µg/band for FOR. Percentage recovery was found to be 100.10±1.039 and 99.96±0.970 for MO and FOR respectively. LOD was found 0.26 µg /band and 0.01 µg /band, LOQ was found 0.78 µg /band and 0.04 µg /band for MO and FOR respectively [14].

Parmar VK et al. developed and validated a method for the simultaneous determination of beclomethasone dipropionate (BDP) and Formoterol fumarate dihydrate (FFD) in marketed rotacap formulation. The method involved HPTLC separation of these two drugs followed by densitometric measurements of their spots at 220 nm. The separation was carried out on Merck HPTLC aluminum sheets precoated with silica gel 60F254 as stationary phase using hexane:ethyl acetate:methanol:formic acid (2.0:2.5:2.0:0.2, v/v/v/v) as mobile phase. The linearity was found to be in the range of 2.4–8.4 ng/spot and 80–280 µg/spot for BDP and FFD, respectively. R_f values were found to be 0.81 ±0.02 and 0.17± 0.02 for MO and FOR, respectively. The robustness of method was assessed using experimental design and results were analyzed by statistical and graphical approaches. Rotacaps formulation containing BDP (200/400 mg) and FFD (6 mg) were successfully quantified using the proposed methods. The proposed method can be used as sensitive, precise, accurate and robust methods for quantification of BDP and FFD in Rotacaps [15].

HPLC (High Performance Liquid Chromatography)

Merey HA et al. developed and validated high performance liquid chromatographic method for the separation and determination of MO and FOR using reversed phase C18 column with isocratic elution. The mobile phase composed of methanol: 0.5% ammonium acetate (pH 5.7 adjusted by glacial acetic acid), (80:20, v/v) at a flow rate of 1.0 mL/min. Quantitation was achieved at 220 nm by UV detection. Retention times for MO and FOR were found to be 6.925

min and 2.717 min respectively. The obtained results were statistically compared with those obtained by the reported methods, showing no significant difference with respect to accuracy and precision at $P_{value} = 0.05$ [16].

Parmar VK et al. developed and validated a method based on HPLC separation of the Beclomethasone dipropionate (BDP) and Formoterol fumarate dihydrate (FFD) on the reversed phase enable HPLC Analytical C18 G 120A° (250 X 4.6 mm, 5 µm) column at ambient temperature using a mobile phase consisting of methanol: acetonitrile: phosphate buffer (65:25:10, v/v/v) adjusted to pH 3.6 using orthophosphoric acid. Quantitation was achieved with UV detection at 220 nm based on peak area with linear calibration curves at concentration ranges of 10–200 and 0.3–6.0 µg/mL for BDP and FFD, respectively. The robustness of method was assessed using experimental design and results were analyzed by statistical and graphical approaches. Rotacaps formulation containing BDP (200/400 mg) and FFD (6 mg) were successfully quantified using the proposed methods. The proposed method can be used as sensitive, precise, accurate and robust methods for quantification of BDP and FFD in Rotacaps [17].

Appala SR et al. developed a simple, rapid and reproducible high performance reverse phase liquid chromatographic method has been developed for quantitative estimation of Formoterol in rotacap using a C-8 column and UV detection at 254 nm. The isocratic elution was used to quantify the analyte. The samples were chromatographed on C-8 column and the mobile phase was ammonium acetate: Acetonitrile (80:20, w/v) was pumped at 1 mL/min. The method was linear between 30-180 µg mL^{-1}, statistically validated for its linearity, precision and accuracy. The intra day and inter day variation was found to be less than 2% showing high precision of the assay method. It was found that the excipients in the commercial rotocaps did not interfere with the method [18].

Shah BD et al RP-HPLC method developed and validated (as per ICH guidelines) for estimation of Tiotropium Bromide & Formoterol Fumarate in Metered Dose Inhalation dosage form. adequate separation for Tiotropium Bromide & Formoterol Fumarate was achieved on a Waters Spherisorb C8, 150 mm x 4.6 mm, 5 nm column at 30 °C temperature by using Ammonium dihydrogen phosphate (pH 3.0): Acetonitrile: Methanol (45:30:25 v/v) as mobile at a flow rate of 1.0 ml/min, and UV detection at 235 nm. In the proposed study the specificity of the method was determined by assessing interference from the placebo & diluent .There were no other co eluting, interfering peaks from excipients, impurities found and the method was specific for estimation of Tiotropium Bromide & Formoterol Fumarat. The linearity of the proposed method was investigated in the range of 0.55-1.65 µg/mL (R^2 = 0.9961) for Tiotropium Bromide and 0.30-0.90 µg/mL (R^2 = 0.9952) for Formoterol Fumarate. Accuracy determined by recovery study was found to be 82.1% - 88.1% w/w for Tiotropium Bromide & 98.7% - 100.7% w/w for Formoterol Fumarate. Assay results of Metered Dose Inhalation dosage form was found to be 99.71% w/w for Tiotropium Bromide & 103.94% w/w for Formoterol Fumarate (for batch 9+6/F001) & 100.59% w/w for Tiotropium Bromide & 96.98% w/w for Formoterol Fumarate (for batch 9+6/F002) [19].

Pai N et al. A reverse phase high performance liquid chromatography method was developed for the simultaneous estimation of Formoterol Fumarate and Budesonide in pressurised metered dose inhaler. The separation was achieved by octadecyl silica gel column (C18) and buffer of sodium dihydrogen phosphate and decane sulphonic acid in combination with acetonitrile as eluent, at a flow rate of 2 ml/min. Detection was carried out at 220 nm. Method was validated as per ICH guidelines and found to be stable, indicating its usefulness for analysis of said drug combination [20].

Gowekar NM et al. developed and validated a simple isocratic reversed phase high performance liquid chromatographic (RP-HPLC) method has been developed for the simultaneous determination of Formoterol fumarate dehydrate and Fluticasone propionate in dry powder inhalation formulation. The separation was achieved by HiQSil C18 HS (250 × 4.6mm , i.d 5µm) column, acetonitrile: 0.01 M ammonium dihydrogenphosphate buffer pH 3.5 adjusted with *o*-phosphoric acid (80: 20 v/v) as mobile phase, at a flow rate of 1 mL/min. The detection was carried out at 215 nm. Retention time of Formoterol fumarate dihydrate and Fluticasone propionate was found to be 4.892 and 9.183 min, respectively. Linearity for Formoterol fumarate dehydrate and Fluticasone propionate were in the range of 2.4-7.8 µg/mL and 10-90 µg/mL, respectively. The mean % recoveries obtained for Formoterol fumarate dehydrate and Fluticasone propionate was found to 99.48 % and 99.54 %, respectively. Developed method was found to be accurate, precise, selective and rapid for simultaneous determination of Formoterol fumarate dihydrate and Fluticasone propionate in dry powder inhalation formulation [21].

Trivedi RK et al developed a stability-indicating reversed-phase high performance liquid chromatography (RP-HPLC) method was developed for the simultaneous determination of Formoterol fumarate (FOR), Tiotropium bromide (TRI), and Ciclesonide (CLS) in a pulmonary drug product. The desired chromatographic separation was achieved on the Zorbax SB C8, 5 µm (150 x 4.6 mm) column, using gradient elution at 230 nm detector wavelength. The optimized mobile phase consisted of a 0.2 % v/v perchloric acid as solvent-A and acetonitrile as solvent-B.

The developed method separated FOR, TRI, and CLS in the presence of its five unknown degradation products within 10 minutes. The stability-indicating capability was established by forced degradation experiments and the separation of unknown degradation products. The developed RP-HPLC method was validated according to ICH guidelines. This validated method was applied for the simultaneous estimation of FOR, TRI, and CLS in commercially available Triohale® pMDI (Pressurized Metered-Dose Inhaler) samples. Furthermore, this method can be extended for individual estimation of FOR, TRI, and CLS in various commercially available pulmonary dosage forms [22].

Raval PP et al. developed a Reversed Phase Ion Pair HPLC method has been developed and validated for the estimation of Fluticasone propionate in nasal preparation and in combination with Formoterol fumarate in DPI (Dry Powder Inhalation). This method was developed using ACE C18 Column (150 mm× 4.6 mm id, 5µm) and Acetonitrile: 10 mM Sodium di-hydrogen ortho phosphate buffer containing 10 mM Decane sulphonic acid sodium salt (50:50 v/v) as the mobile phase at 1ml/min flow rate with detection at 220 nm. Decane sulphonic acid sodium salt proved to be better than other anionic ion pairing agents. This method was validated as per ICH guideline. The method was linear in the concentration of 1.5-12 µg/mL for Formoterol and 25-200 µg/mL for Fluticasone with a correlation coefficient of 0.999 each. The LOQ for Formoterol and Fluticasone was found to be 0.0244 µg/ml and 0.7445 µg/ml respectively. The method successfully separated both the drugs from potential degradation products in stress samples with adequate peak purity establishing specificity and stability indicating nature of the method. Both the drugs degraded under photolytic condition; but Fluticasone degraded more and yielded there degradation peaks which further increased in presence of Formoterol. Both the drugs did not show degradation under thermal, thermal-humidity conditions. Fluticasone was more susceptible to alkaline hydrolysis giving two degradation peaks as compared to acidic hydrolysis when yielded one degradation peak. All method validation parameters were within the acceptance criteria as per ICH guideline. The method is deemed to be stability indicating as it separates both the drugs from likely degradation products [23].

Srinivasrao K al. developed a simple, precise, accurate, and stability-indicating method and validated for analysis of Formoterol fumarate and Mometasone furoate in metered dose inhalation formulations. Separation was achieved on a reversed-phase C18 column (150 mm × 4.6 mm i.d., 5 µm) using a mobile phase consisting of Sodium dihydrogen orthophosphate buffer/acetonitrile (50:50, v/v) at a flow rate of 1.0 mL/min and UV detection at 220 nm. This method is validated according to United States Pharmacopeia requirements for new methods, which include accuracy, precision, selectivity, robustness, and linearity and range. This method shows enough selectivity, accuracy, precision, and linearity and range to satisfy Federal Drugs Administration/International Conference on Harmonization regulatory requirements. Linearity were found to be over the range 0.01-0.20 mg/mL of Formoterol fumarate with R^2=0.999 and 0.40-6.00 mg/mL of mometasone furoate with R^2=0.999. The average recovery of the method is 99.9% of Formoterol fumarate with a % RSD of 1.94% and 101.5% of mometasone furoate with a % RSD of 0.81%. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters and by changing analytical operators has proven that the method is robust and rugged [24].

Kale NR et al. developed a stability indicating RP-HPLC method was developed and validated for the simultaneous determination of Formoterol Fumarate (FF) (6 µg) and Budesonide (BUD) (400 µg) in metered dose inhaler formulation. The chromatographic separations was carried out with Waters alliance 2695 separation module, 2996 PDA detector and empower software and Shimadzu LC HCT 10 with UV detector and LC Solution Software instruments, using Hypersil BDS C-18, 150 x 4.6 mm, 5 µ column and gradient elution with NaH2PO4.H2O buffer (pH 3.1 with OPA) and acetonitrile as mobile phase at a flow rate of 1.5 ml/min. The detection was carried out on dual wavelength detector at 214 nm and 247 nm for FF and BUD respectively. The detector response is linear from 0.3-0.9 µg/ml and 20-60 µg/ml concentrations for FF and BUD respectively. The linear regression equation was found to be $y = 56974x - 170.74$ ($r^2 = 0.9997$) for FF and $y = 28487x - 44972$ ($r^2 = 0.9994$) for BUD. The stability indicating capability was established by forced degradation experiments. The method was satisfactorily validated as per the ICH guidelines [25].

Gujarati PZ et al. developed A simple, specific, accurate, and stability indicating reversed-phase high-performance liquid chromatographic method was developed for the simultaneous determination of Formoterol fumarate and Mometasone furoate using a Enamal C18 column and a mobile phase composed of acetonitrile: 0.05 M orthophosphoric acid: methanol (60:30:10 v/v), pH 3. The retention times of Formoterol fumarate and Mometasone furoate found to be 1.68 min and 7.17 min, respectively. Linearity was established for of Formoterol fumarate and Mometasone furoate in the range of 3-9 µg/ ml and 100-300 µg/ml, respectively. The percentage recoveries of Formoterol fumarate and Mometasone furoate were found to be in the range of 98.66-99.31%. Both the drugs were subjected to acid and base hydrolysis, oxidation, photolytic, and thermal degradation conditions. The degradation products of Formoterol fumarate and Mometasone furoate were well resolved from the pure drug with significant differences in their retention time values. This method can be successfully employed for simultaneous quantitative analysis of Formoterol fumarate and Mometasone furoate in bulk drugs and formulations [26].

Jain HK et al. developed A simple, specific, accurate, and stability indicating reversed-phase high-performance liquid chromatographic method for the simultaneous determination of Formoterol fumarate and Mometasone furoate using a Enamal C18 column and a mobile phase composed of acetonitrile: 0.05 M orthophosphoric acid: methanol (60:30:10 v/v), pH 3. The retention times of Formoterol fumarate and Mometasone furoate found to be 1.68 min and 7.17 min, respectively. Linearity was established for of Formoterol fumarate and Mometasone furoate in the range of 3-9 μg/ ml and 100-300 μg/ml, respectively. The % recoveries of Formoterol fumarate and Mometasone furoate were found to be in the range of 98.66-99.31%. Both the drugs were subjected to acid and base hydrolysis, oxidation, photolytic, and thermal degradation conditions. The degradation products of Formoterol fumarate and Mometasone furoate were well resolved from the pure drug with significant differences in their retention time values. This method can be successfully employed for simultaneous quantitative analysis of Formoterol fumarate and Mometasone furoate in bulk drugs and formulations [27].

El-Bagary RI et al. developed and validated two simple, selective and precise stability-indicating reversed-phase liquid chromatographic methods for the determination of Mometasone furoate in two binary mixtures, with Formoterol fumarate (Mixture 1) and salicylic acid (Mixture 2). Also, a forced degradation study of Mometasone furoate was carried out including acid and alkali hydrolysis, oxidation, thermal and photo-degradation. For mixture 1, the method was based on isocratic elution using a mobile phase consisting of (Acetonitrile: 3 mM Sodium lauryl sulfate) (60:40, v/v) at a flow rate of 1 mL/min. Quantitation was achieved applying dual wavelength detection where Mometasone furoate and its degradation products were detected at 247 nm and Formoterol fumarate and its degradation product were detected at 214 nm at 30 °C. For mixture 2 and for the forced degradation study, separation was based on isocratic elution of Mometasone furoate, its degradation products and salicylic acid on a reversed phase C8 column using a mobile phase consisting of acetonitrile: water: methanol: glacial acetic acid (60:30:10:0.1, v/v) at a flow rate of 2 mL/ min. Quantitation was achieved with UV detection at 240 nm. In addition, products from alkaline forced degradation of Mometasone furoate were verified by LC–MS. Linearity, accuracy and precision were found to be acceptable over the concentration range of 10–800 μg/mL and 5–60 μg/mL for Mometasone furoate and Formoterol fumarate, respectively and over the concentration range of 5–320 μg/mL and 20–1280 μg/mL for Mometasone furoate and salicylic acid [28].

Graham SJ et al developed a HPLC method for determination of Formoterol using a 3-µm octyl bonded phase column, 4.6 × 50 mm, and a mobile phase of water- acetonitrile- trifluoroacetic acid in a ratio of 800:200:0.5 (v/v/v) at a flow rate of 2.0 ml/min. The LOQ was found to be 0.02 µg/mL based on injection of 200-µL aliquots and the average between day co efficient of variation [29].

Patil AS et al. developed a sensitive stability indicating reverse phase high performance liquid chromatographic method was developed for the simultaneous determination of Formoterol fumarate and Ciclesonide in dry powder inhaler. The chromatographic separation was achieved on Hypersil BDS C8 250 × 4.6 mm, 5 um column using a mobile phase consisting of 0.1% orthophospheric acid and acetonitrile in the ratio of 35:65 (v/v), at a flow rate of 2.0 mL min−1. The column compartment temperature was set at 40°C. The typical HPLC chromatograms were extracted at 214 nm using photodiode array detector (PDA). The described method shows excellent linearity over a range of 3.6 µg mL−1 to 1.1 ng mL−1 for Formoterol fumarate and 120 µg mL−1 to 34 ng mL−1 for Ciclesonide. The correlation coefficient for Formoterol fumarate and Ciclesonide was found to be 0.9998 and 0.9999 respectively. The limit of detection and limit of quantification for Formoterol fumarate was 0.33 ng mL−1 and 1.1 ng mL−1 while for Ciclesonide 10.2 ng mL−1 and 34 ng mL−1, respectively. The proposed method was found to be very sensitive and accurate for the determination of Formoterol Fumarate and Ciclesonide in dry powder inhaler [30].

Apako SO et al. developed a stability-indicating reversed-phase high performance liquid chromatographic (HPLC) method has been developed and validated for the assay of Formoterol fumarate and the related substances, namely, Formoterol fumarate desformyl and Formoterol fumarate acetamide analogs, in the active pharmaceutical ingredient. The separation was achieved by isocratic elution using an Alltech Alltima C18 (150×4.6 mm) column, a mobile phase consisting of ammonium acetate (50 mM; pH 5.0)–ethanol (65:35, v/v), a flow rate of 1.0 ml/min and UV detection at 242 nm. The detection and quantitation limits were 0.03 and 0.08 µg/ml, respectively, while the linear range of detection was between 0.03 and 255 µg/ml. Comparative determinations of Formoterol fumarate in three lots of bulk drugs using the proposed HPLC method and the standard potentiometric titration method of pharmacopoeia show that both methods are equivalent for pure drug substance assay. However, the HPLC method allowed the separation and quantitation of the impurities not achievable with the official methods in the bulk drugs. This study shows that the proposed method is accurate, linear, and sensitive as stability indicating assay method for Formoterol fumarate in the bulk drug [31].

Bio Analytical Methods

Mezzarino M et al. developed and validated direct LC–ESI-MS/MS. Since 1992, Formoterol is included in the prohibited list of doping substances and methods, presently reviewed and updated by the World Anti-Doping Agency. Recently a threshold value (40 ng/mL) has been established to differentiate between the prohibited (oral) and the permitted (inhalatory) administration of Formoterol to athletes. This paper considers the urinary excretion profile of Formoterol and its main metabolites after inhalation of different doses of two of the most used medicaments, available in Italy, containing Formoterol fumarate bihydrate (12 and 36 µg twice a day of Foradil® or 9 and 27 µg twice a day of Symbicort®), focusing also on the effects, on the measured levels of Formoterol, of potential alteration processes (thermal and/or microbiological) that may take place after the collection of the urine samples. Urine sample preparation included an enzymatic hydrolysis and a dilution step. Detection of analytes was performed by a newly developed and validated direct LC–ESI-MS/MS procedure, using a triple quadrupole mass spectrometer under positive ion electro-spray ionization conditions and selected reaction monitoring acquisition mode. The results showed the capability and suitability of the direct LC–ESI-MS/MS analysis for the quantitative confirmation analysis of Formoterol in urine samples. The data from the analysis of the urine samples obtained in the excretion studies showed that Formoterol is excreted mainly as unmodified drug and to a lesser degree as O-demethylated metabolite. The urinary levels of Formoterol (40–60%) and its metabolites (O-demethylated metabolite 5–25%; glucuronide metabolites 25–40%) vary significantly depending both on the administered drug formulation and the subject tested. The maximum urinary concentration reached in this study was 15 ng/mL (free + glucuronide), that is significantly lower than the threshold value fixed to report an adverse analytical finding. Finally, our results also showed that Formoterol is stable for at least 4 weeks in urine samples correctly collected and stored [32].

Campestrni J et al. developed and validated an automated high-performance liquid chromatography (HPLC) method for the determination of Formoterol in human plasma with improved sensitivity. Formoterol and CGP 47086, the internal standard, were extracted from plasma (1 ml) using a cation-exchange solid-phase extraction (SPE) cartridge. The compounds were eluted with pH 6 buffer solution–methanol (70:30, v/v) and the eluate was further diluted with water. An aliquot of the extract solution was injected and analyzed by HPLC. The extraction, dilution, injection and chromatographic analysis were combined and automated using the automate (ASPEC) system. The chromatographic separations were achieved on a 5 µm, Hypersil ODS analytical column (200 mm×3 mm I.D.), using (pH 6 phosphate buffer, 0.035

M+20 mg/l EDTA)–MeOH–CH3CN (70:25:5, v/v/v) as the mobile phase at a flow-rate of 0.4 ml/min. The analytes were detected with electrochemical detection at an operating potential of +0.63 V. Intra-day accuracy and precision were assessed from the relative recoveries of calibration/quality control plasma samples in the concentration range of 7.14 to 238 pmol/l of Formoterol base. The accuracy over the entire concentration range varied from 81 to 105%, and the precision (C.V.) ranged from 3 to 14%. Inter-day accuracy and precision were assessed in the concentration range of 11.9 to 238 pmol/l of Formoterol base in plasma. The accuracy over the entire concentration range varied from 98 to 109%, and precision ranged from 8 to 19%. At the limit of quantitation (LOQ) was found to be 11.9pmol/l for inter-day measurements. The %recovery was found to be 109%. As shown from intra-day accuracy and precision results, favorable conditions (a newly used column, a newly washed detector cell and moderate residual cell current level) allowed us to reach a LOQ of 7.14 pmol/l of Formoterol base (3 pg/ml of Formoterol fumarate dihydrate). Improvement of the limit of detection by a factor of about 10 was reached as compared to the previously described methods. The method has been applied for quantifying Formoterol in plasma after 120 μg drug inhalation to volunteers. Formoterol was still measurable at 24 hr post-dosing in most subjects and a slow elimination of Formoterol from plasma beyond 6–8 h after inhalation was demonstrated for the first time thanks to the sensitivity of the method [33].

Kamimura H developed method for the quantitative determination of the β-stimulant Formoterol in urine using a gas chromatograph—mass spectrometer. Formoterol can be analyzed after addition of a deuterium-labelled internal standard and conversion to a mixed bispentafluoropropionyl-methyl derivative for selected ion monitoring. The detection limit was 5ng/ml. Urinalysis after the oral administration of Formoterol fumarate, using a combined enzymic hydrolysis method, revealed that the drug was conjugated with glucuronic acid in rats, dogs and humans [34].

Nadarassan DK et al. developed and validated a novel high-performance liquid chromatography (HPLC) assay for the estimation of Formoterol in urine samples. A solid phase extraction (SPE) using Oasis HLB was optimized to isolate Formoterol from a urine matrix followed by HPLC with UV detection. This extraction procedure concentrated the final analyte forty times so that UV detection can be used to determine even a low concentration of Formoterol in urine samples. The urinary assay was performed in accordance with FDA and ICH regulations for the validation of bioanalytical samples. The samples were injected onto a C18 Spherisorb® (250 mm × 4.6 mm × 5 μm) analytical column maintained at 30 °C. The mobile phase consisted of 5 mM of potassium dihydrogen orthophosphate buffer (adjusted to pH 3 with ortho phosphoric acid):acetonitrile (ACN) (70:30, v/v), and the Formoterol peak was detected at wavelength 214 nm. The extraction recovery of Formoterol from the urine sample was >95%. The calibration curve was linear ($r2$ = 0.99) over Formoterol concentrations ranging from 1.5 to 25 ng/mL (n=6). The method had an accuracy of >92% and intra and inter-day precision CV% of <3.9% and <2.2%, respectively, at three different concentrations low, medium and high (10, 15, 20 ng/mL). The limit of quantification (LOQ) for Formoterol was found to be 1.50 ng/mL. The accuracy and precision at the LOQ level were 95% and %CV <3.7% (n = 10), respectively. The method reported is simple, reliable, precise, and accurate and has the capacity to be used for determination of Formoterol in urine samples [35].

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