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A Systematic Review On Analytical Methods For Empagliflozin And Metoprolol Succinate

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Abstract:

Empagliflozin is a relatively new drug that, as an inhibitor of the sodium–glucose cotransporter 2 (SGLT2), causes increased urinary glucose excretion and thus contributes to improved glycaemic control, better glucose metabolism, reduced glucotoxicity and insulin resistance. Although its original use was to induce a hypoglycaemic effect in patients with type 2 diabetes mellitus. **Metoprolol Succinate** is the succinate salt form of metoprolol, a cardio selective competitive beta-1 adrenergic receptor antagonist with antihypertensive properties and devoid of intrinsic sympathomimetic activity. Metoprolol succinate antagonizes beta 1-adrenergic receptors in the myocardium. This review summarizes various analytical methods development for both drugs, including: UV, HPLC, HPTLC, LC-MS/MS. This method has been validation for parameters such as: linearity, wavelength, solvent, mobile phase, flow rate, retention time and column.

Keywords : Empagliflozin, Metoprolol Succinate, Analytical methods, Validation parameters

1. Introduction

Empagliflozin is a relatively new drug that, as an inhibitor of the sodium–glucose cotransporter 2 (SGLT2), causes increased urinary glucose excretion and thus contributes to improved glycemic control, better glucose metabolism, reduced glucotoxicity and insulin resistance. Although its original use was to induce a hypoglycaemic effect in patients with type 2 diabetes mellitus.

Metoprolol Succinate is the succinate salt form of metoprolol, a cardio selective competitive beta-1 adrenergic receptor antagonist with antihypertensive properties and devoid of intrinsic sympathomimetic activity. Beta-1 adrenergic receptors in the myocardium are antagonized by metoprolol succinate. This review summarizes various analytical methods development for both drugs, including: UV, HPLC, HPTLC, LC-MS/MS. This method has been validation for parameters such as: linearity, wavelength, solvent, mobile phase, flow rate, retention time and column

This review article summarizes and critically evaluates the various analytical methods reported for the estimation of Empagliflozin and Metoprolol Succinate in bulk and pharmaceutical dosage forms. The methods discussed include UV spectrophotometry, High-Performance Liquid Chromatography (HPLC), High-Performance Thin Layer Chromatography (HPTLC), and Liquid Chromatography–Mass Spectrometry (LC-MS/MS). These techniques have been widely investigated due to their sensitivity, specificity, and suitability for routine quality control analysis.

2. Mechanism of action

Empagliflozin, an SGLT-2 inhibitor, is used in the management of type 2 diabetes and associated cardiovascular complications. Metoprolol Succinate, a cardio-selective β1-blocker, is employed in hypertension, angina, myocardial infarction, and arrhythmias. Due to their therapeutic importance, the development of robust analytical methods is essential.

UV spectrophotometric methods reported for both drugs demonstrate simplicity and cost-effectiveness, with λ_{max} values ranging between 220–275 nm depending on solvent systems. HPLC remains the most widely used technique, with reverse-phase C18 columns and mobile phases comprising methanol, acetonitrile, buffers, or combinations thereof. Methods show excellent linearity, short retention times, and precision suitable for routine assays.

LC-MS/MS methods offer superior sensitivity and selectivity, making them ideal for pharmacokinetic and bio analytical applications (1). Meanwhile, HPTLC provides a rapid and economic alternative for simultaneous estimation in combined dosage forms.

Overall, the literature demonstrates that validated analytical methods for Empagliflozin and Metoprolol Succinate fulfill regulatory requirements in terms of accuracy, precision, linearity, robustness, and

specificity. The availability of multiple techniques allows flexibility depending on laboratory resources and analytical requirements.

Metoprolol competes with adrenergic neurotransmitters such as catecholamines for binding at beta (1)-adrenergic receptors in the heart. Beta (1)- receptor blockade results in a decrease in heart rate, cardiac output, and blood pressure .

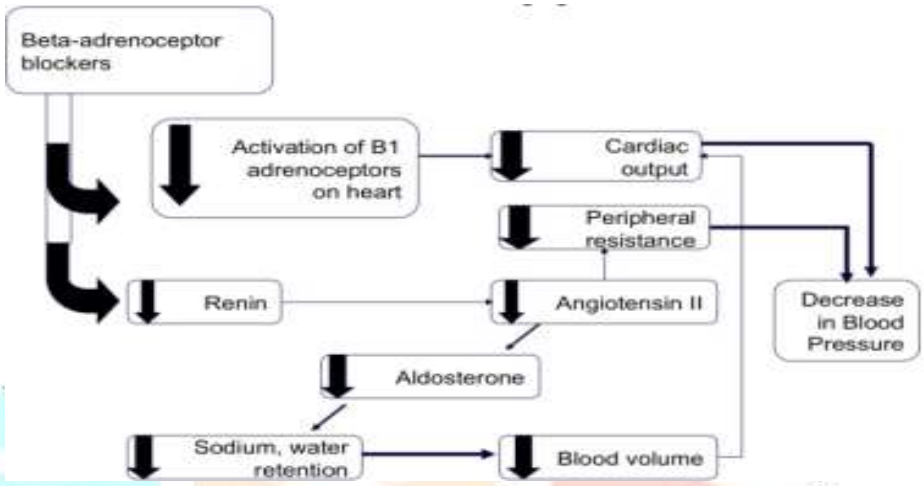

Figure.1: Mechanism of action of metoprolol succinate

3. Analytical Methods

3.1.1 UV Spectrophotometric Methods

UV spectrophotometry remains one of the most widely employed analytical techniques for the estimation of Empagliflozin alone and in combination with other antidiabetic agents. Its advantages include simplicity, cost-effectiveness, rapid analysis, and minimal sample preparation. Over the years, multiple UV methods have been developed, each utilising different solvents, wavelength maxima, and linearity ranges tailored to specific analytical needs.

The earliest methods reported simple quantification of Empagliflozin using water–methanol mixtures, establishing λ_max values around 223–224 nm with narrow linearity ranges suitable for bulk drug analysis. Subsequent studies expanded the analytical scope, offering improved linearity ranges up to 25–30 mg/mL and replacing organic solvents with greener alternatives such as distilled water or 0.1 M urea to enhance environmental sustainability.

Simultaneous estimation methods for Empagliflozin in fixed-dose combinations—particularly with Metformin hydrochloride and Linagliptin—have further diversified UV approaches. These studies established distinct λ_{max} values for each drug component, enabling accurate multi-component analysis using

various calibration models. Green analytical chemistry principles were incorporated in some methods through chemometric techniques and the use of eco-friendly solvents.

Novel spectrophotometric approaches have also introduced both UV and visible region quantification, expanding detection capabilities and offering alternative wavelength maxima (e.g., 247 nm for ethanol-based analysis). These developments collectively highlight the versatility and robustness of UV spectrophotometric techniques for routine quality control of Empagliflozin-containing formulations (2).

A concise summary of key UV spectrophotometric methods reported in literature is provided below:

a) Development and Validation of a Simple UV-Spectrophotometric Method for the Determination of Empagliflozin
b) UV Spectroscopic Method Development and Validation for Empagliflozin in Bulk and Tablet Dosage Forms
c) Formulation and Verification of UV-Spectral Analytical Methods for the Simultaneous Measurement of Empagliflozin and Metformin Hydrochloride in Combination Products Development and Validation of a Novel Analytical Method for Empagliflozin and Metformin Hydrochloride in Bulk and Pharmaceutical Dosage Forms Using Four Distinct UV-Based Simultaneous Estimation Strategies (3).
d) Green Analytical methods based on chemometrics and UV spectroscopy for the simulation estimation of Empagliflozin and Linagliptin.
e) Innovative UV–Visible Spectrophotometric Techniques for the Determination of Empagliflozin, a Type 2 Antidiabetic Agent, in Bulk Drug and Pharmaceutical Formulations Development and validation of UV spectrophotometric method for simultaneous estimation of Empagliflozin and Linagliptin in bulk drugs and pharmaceutical dosage form
f) Development and Validation of an Analytical Method for Empagliflozin in Bulk Drug and Pharmaceutical Dosage Forms Using UV Spectroscopy

3.1.2 High-Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography (HPLC) is one of the most reliable and extensively used analytical techniques for the quantification of Empagliflozin in bulk drug, pharmaceutical formulations, biological matrices, and combination therapies. Owing to its high sensitivity, precision, and specificity, HPLC offers significant advantages over UV spectrophotometry, particularly for stability studies, impurity profiling, and simultaneous multi-component analysis (4).

Most reported HPLC methods for Empagliflozin utilize reversed-phase C18 columns with various dimensions and particle sizes to achieve optimal separation. UV detection remains the most commonly employed detection technique, with λ_max values typically ranging from 222 to 270 nm. Mobile phases

reported in the literature encompass a wide variety of solvent systems including water, methanol, acetonitrile, orthophosphoric acid, TFA, and phosphate buffers, enabling effective separation under diverse chromatographic conditions. Flow rates predominantly range between 0.7 to 1.2 mL/min, depending on column characteristics and the complexity of the analyte mixture.

Several methods focus on the estimation of Empagliflozin alone for routine quality control, while others offer validated simultaneous estimation approaches with companion drugs such as Metformin, Pioglitazone, and Linagliptin. Stability-indicating HPLC methods have also been widely developed, demonstrating the capability of Empagliflozin to undergo degradation under stress conditions including oxidation, acid/base hydrolysis, and photolysis. These studies employ DAD, PAD, or UV detectors to confirm peak purity and method robustness. Additionally, a few advanced methods address impurity profiling and pharmacokinetic applications, extending the relevance of HPLC techniques beyond basic assay (5).

3.1.3 LC–MS/MS Methods

Liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) represents the most advanced analytical platform for the quantitative and qualitative determination of Empagliflozin, particularly in biological matrices where high sensitivity and specificity are essential. The technique provides excellent detection limits, rapid analysis, and robust selectivity, making it highly suitable for pharmacokinetic, bioequivalence, and therapeutic drug monitoring studies (6).

LC–MS/MS methods developed for Empagliflozin commonly employ ultra-performance liquid chromatography (UPLC) or high-resolution MS systems, typically using short C18 columns (50 × 2.1 mm) with sub-2-micron particle sizes to achieve superior resolution and faster chromatographic run times. Mobile phases generally consist of volatile buffers such as formic acid or ammonium acetate in combination with acetonitrile, ensuring optimal ionization efficiency under electrospray ionization (ESI) conditions. Flow rates are maintained between 0.2 and 1.0 mL/min depending on the column specifications and instrument sensitivity requirements (6).

Most LC–MS/MS methods report extremely low limits of detection and wide linearity ranges, enabling accurate quantification of Empagliflozin at trace levels in plasma, urine, and formulation matrices. Sample preparation frequently involves protein precipitation or mixed-mode solid-phase extraction (SPE), the latter providing superior cleanup for complex biological samples and improving method sensitivity .

Simultaneous quantification methodologies have been developed extensively for Empagliflozin in combination with antidiabetic agents such as Metformin, Linagliptin, and other SGLT2 inhibitors (canagliflozin, dapagliflozin). These methods demonstrate rapid retention times—often below 3 minutes for Empagliflozin—facilitating high-throughput analysis suitable for clinical studies. UPLC-MS/MS in

particular has shown excellent performance, with linearity ranges extending as low as 0.02 µg/mL for Empagliflozin, highlighting the enhanced sensitivity of the technique.

Advanced LC–MS/MS approaches have also been applied to structural elucidation and degradation studies. Methods incorporating characterization of degradation products provide insights into impurity formation and stability behaviour, supporting formulation development and regulatory submissions. Overall, LC–MS/MS remains the most powerful and sensitive analytical tool for Empagliflozin determination, offering unparalleled performance for both routine and research applications .

3.1.4 High-Performance Thin-Layer Chromatography (HPTLC)

High-Performance Thin-Layer Chromatography (HPTLC) has been widely employed for the qualitative and quantitative assessment of empagliflozin both as a single component and in combination with other antidiabetic drugs. These methods offer advantages such as simplicity, cost-effectiveness, parallel sample processing, and suitability for stability-indicating analysis. (7)

One of the reported HPTLC methods focuses on the development of a stability-indicating assay for empagliflozin in pharmaceutical dosage forms. The method utilizes silica gel 60 F254 HPTLC plates as the stationary phase and a toluene:methanol (7:3) mobile phase system. Detection is carried out using UV detection at 240 nm, and empagliflozin exhibits an Rf value of 0.65, demonstrating good resolution and peak characteristics .

Another study reports a novel stability-indicating HPTLC method for the simultaneous estimation of metformin hydrochloride and empagliflozin in bulk and marketed formulations. The chromatographic separation is achieved on silica gel aluminium plates employing a mobile phase consisting of ammonium acetate:isopropyl alcohol:triethylamine (4:6:0.1). The method allows effective separation with Rf values of 0.82 ± 0.02 for metformin hydrochloride and 0.50 ± 0.02 for empagliflozin, using UV detection at 242 nm.

Collectively, these HPTLC methods demonstrate effective chromatographic resolution, reproducibility, and stability-indicating capability, making them valuable tools for routine quality control and formulation analysis of empagliflozin and its combinations .

3.2 Metoprolol Succinate

3.2.1 UV Spectrophotometric Methods

UV spectrophotometry remains one of the most widely adopted analytical tools for the quantification of metoprolol succinate in bulk and pharmaceutical dosage forms. Owing to its simplicity, cost-effectiveness, and rapid analysis, several researchers have reported validated UV-based methods for both single and multi-component formulations.

Initial studies focused on the estimation of metoprolol succinate as a single component, with λmax values commonly observed around 221–223 nm, depending on solvent systems. One method reported λmax at 222 nm, employing hydrochloric acid, water, and methanol as solvents, with a linearity range of 5–25 µg/mL . Similarly, another spectroscopic method demonstrated λmax at 223 nm using chloroform and an ethanol–water mixture, maintaining the same linearity range . An additional method, using methanol as solvent, identified λmax at 275 nm and a broader linearity of 50–250 µg/mL, suitable for higher concentration analysis . A separate study using methanol reported λmax at 222 nm, with improved sensitivity over the linearity range 2–16 µg/mL

Numerous UV spectrophotometric methods have also been developed for the simultaneous estimation of metoprolol succinate with other antihypertensive agents. For instance, a combined assay with olmesartan medoxomil employed λmax values of 221 nm (metoprolol succinate) and 257 nm (olmesartan), with linearity ranges of 5–25 µg/mL and 4–20 µg/mL, respectively, using water as the solvent . Another method utilized methanol, identifying λmax at 230 nm for metoprolol succinate and 256 nm for olmesartan medoxomil, with linearity ranges of 5–30 µg/mL and 2–12 µg/mL, respectively .

Further multi-component determinations include a validated method for simultaneous estimation of amlodipine besylate and metoprolol succinate, featuring λmax values at 221 nm and 364.6 nm and linearity ranges of 2–20 µg/mL and 1–10 µg/mL, respectively, using water as solvent . Another study reported simultaneous determination of cilnidipine and metoprolol succinate, with λmax values identified at 223.40 nm and 230.60 nm, and linearity ranges of 10–50 µg/mL and 2–10 µg/mL, respectively, using methanol (8).

Overall, UV spectrophotometric techniques demonstrate excellent precision, sensitivity, and reproducibility for metoprolol succinate estimation, both alone and in combination with other pharmaceutical agents, highlighting their suitability for routine quality control and formulation analysis.

3.2.2 High-Performance Liquid Chromatography (HPLC) Methods

High-performance liquid chromatography (HPLC) has been extensively employed for the quantitative estimation of Metoprolol Succinate in bulk drug, pharmaceutical formulations, and in combination with other antihypertensive agents. Among chromatographic approaches, reverse-phase HPLC (RP-HPLC) remains the most widely adopted technique due to its robustness, reproducibility, and ability to achieve high resolution of analytes under diverse analytical conditions.

Across reported methods, most studies utilized C18 columns of dimensions ranging from 150–250 mm length and 4.6 mm internal diameter with particle sizes of 5–10 µm, offering adequate separation efficiency. Detectors used include UV, PDA/DAD, and UV–VIS systems, with λ_max values varying between 200–280 nm, depending on the analyte and co-formulated drugs .

Mobile phase compositions commonly involved combinations of acetonitrile and buffer systems, such as phosphate buffer, sodium dihydrogen phosphate buffer, or trifluoroacetic acid solutions, typically in ratios ranging from 30:70 to 75:25 (organic:buffer). Flow rates were generally maintained at 1.0 mL/min, ensuring optimum peak shape and reproducibility. Retention times for Metoprolol Succinate varied widely across studies (1.6–5.38 min), influenced mainly by column chemistry, mobile phase strength, and pH.

Method validation results consistently demonstrated excellent linearity, with validated concentration ranges between 0.75–300 µg/mL, depending on the analytical purpose—routine estimation, stability-indicating analysis, or simultaneous multi-drug quantification. Stability-indicating methods efficiently resolved Metoprolol Succinate from its degradation products under forced degradation conditions, confirming the specificity of RP-HPLC approaches (9).

Furthermore, simultaneous HPLC methods have been developed to quantify Metoprolol Succinate alongside commonly co-administered drugs such as Olmesartan Medoxomil, Hydrochlorothiazide, Chlorthalidone, and Azelnidipine. These studies illustrate the versatility of RP-HPLC in multi-component analysis, enabling efficient routine quality control. Columns such as YMC-Pack CN, Zorbax SB Phenyl RP18, and Inertsil ODS-3V demonstrated satisfactory analyte separation in combination drug products. Retention times for co-formulated drugs ranged from 1.6–7.9 min, enabling rapid analysis and improved laboratory throughput (10).

Collectively, the literature highlights the reliability of RP-HPLC as an analytical tool for Metoprolol Succinate. The method's adaptability to various formulations, compatibility with stability testing, and suitability for simultaneous estimation confirm its importance in pharmaceutical analysis (11).

3.2.3 High-Performance Thin Layer Chromatography (HPTLC) Methods

High-performance thin layer chromatography (HPTLC) has emerged as an efficient, economical, and versatile analytical technique for the estimation of Metoprolol Succinate alone and in combination with other antihypertensive agents. Compared to classical TLC, HPTLC offers enhanced resolution, improved reproducibility, and the ability to analyze multiple samples simultaneously, making it valuable for routine quality control of pharmaceutical formulations (12)

Most reported HPTLC methods utilize Silica Gel 60 F254 as the stationary phase, reflecting its widespread acceptance for normal-phase chromatographic separations. In certain studies, RP-18 modified plates were employed to enhance selectivity and achieve better separation of co-formulated drugs, demonstrating the method's adaptability to different polarity requirements (13).

Mobile phase compositions varied considerably across methods depending on the analyte combination. Typical solvent systems included mixtures of toluene, chloroform, methanol, ethyl acetate, and triethylamine, which allowed optimized resolution and well-defined, compact spots. The R_F values for Metoprolol

Succinate ranged widely (0.25–0.70), influenced by differences in mobile phase strength, stationary phase polarity, and co-analytes. In every study, clear separation from companion drugs such as Cilnidipine, Amlodipine Besylate, Hydrochlorothiazide, Telmisartan, and Isosorbide Mononitrate was achieved, indicating good specificity of the optimized HPTLC methods (14).

Detection was typically carried out using UV densitometric scanning, with λ_max values falling within 215–254 nm, depending on the drug mixture. The selected wavelengths ensured optimal sensitivity for Metoprolol Succinate and co-formulated drugs, improving quantification accuracy. The developed methods were validated according to standard analytical performance parameters, including linearity, precision, accuracy, robustness, and specificity. All reported methods demonstrated satisfactory linearity across method-specific concentration ranges and showed minimal interference from excipients or degradation products. (5)

The inclusion of both normal-phase and RP-HPTLC approaches in the literature highlights the flexibility of HPTLC for multi-component analysis. Its capacity for simultaneous processing of many samples, lower solvent consumption, and reduced analysis time makes it an attractive alternative to HPLC, particularly in resource-limited environments. Overall, HPTLC has proven to be a reliable, cost-effective, and efficient technique for the determination of Metoprolol Succinate in various pharmaceutical formulations (5).

Table 1 Summary of work done on Analytical Methods (15).

Drug	UV	HPLC	HPTLC	LC-MS
Empagliflozin	✓	✓	✓	✓
Metoprolol Succinate	✓	✓	✓	×

Drug	UV	HPLC	HPTLC	LC-MS
Empagliflozin	**8**	**14**	**2**	**5**
Metoprolol Succinate	**8**	**9**	**5**	**-**

This review summarizes various analytical methods development for both drugs, including: UV, HPLC, HPTLC, LC-MS/MS. This method has been validation for parameters such as: linearity, wavelength, solvent, mobile phase, flow rate, retention time and column (8).

4. Conclusion

This literature review presents the analytical methods which are available for the determination of the Empagliflozin and Metoprolol succinate in individual form. In this literature review UV Spectroscopy, HPLC, LC-MS/MS and HPTLC methods for the determination of Empagliflozin and Metoprolol succinate. The current review will be very beneficial to researchers in analytical chemistry that develop and validate methods for Empagliflozin and Metoprolol succinate.

This literature review presents the analytical methods which are available for the determination of the Empagliflozin and Metoprolol succinate. AVG Data of empagliflozin in UV method, λmax was 233 nm respectively, linearity was 1-25µg/mL solvent used was methanol or water. Data of Metoprolol succinate in UV method: λ max was 230 nm respectively, linearity was 1-25µg/mL solvent used was methanol or water. In HPLC method, column used was C18 column (250 mm×4.6 mm,5µm), flow rate was 1 mL/min, detector used was PDA and UV, polar mobile phase was used in different ratio and value of retention time changes according to mobile phase ratio, for both drugs. LC-MS method is only used in empagliflozin, column was used C18, polar mobile phase, flow rate was different 0.7,0.8,0.9 mL/min and linearity also founded different value like 1.5,2,5,10 µg/mL, flow rate was 1.0ml/min, retention time also has been various. In HPTLC, commonly used stationary phase was silica gel 60F254 plates, with value of Rf changes according to mobile phase ratio in both.

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