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"A REVIEW ON CURRENT PERSPECTIVE ON ANALYTICAL METHODOLOGIES FOR SIMULTANEOUS ESTIMATION OF EMPAGLIFLOZIN & FUROSEMIDE IN COMBINATION"

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ABSTRACT
Combinations of empagliflozin, a sodium–glucose co-transporter 2 (SGLT2) inhibitor, and furosemide, a potent loop diuretic, are gaining prominence in the management of comorbid diabetes and heart failure. Thus reliable analytical methodologies are essential to ensure the safety, efficacy, and quality of such formulations. This review provides a comprehensive perspective on the current advancements in analytical techniques employed for the simultaneous estimation of empagliflozin and furosemide in combined dosage forms. Various spectrophotometric, chromatographic, and hyphenated approaches are critically evaluated with respect to sensitivity, selectivity, accuracy, and regulatory compliance. Emphasis is given to method development strategies, validation parameters, and challenges associated with it. By providing a consolidated overview, our aim was to assess the diuretic and natriuretic effect of empagliflozin in combination with loop diuretics.

Keywords: Empagliflozin, Furosemide, Simultaneous Estimation, Fixed-Dose Combination, Analytical Method Development, Spectrophotometry, Chromatography, Method Validation, Regulatory Compliance

INTRODUCTION
The number of heart failure (HF) patients is on the rise worldwide, and there are 6.7 million HF patients in the United States [1 2]. Inhibitors of sodium–glucose cotransporter 2 (SGLT2i) reduce the risk of hospitalization for heart failure (HF). We aimed to examine the effect of empagliflozin on change of diuretics dose in outpatient HF patients [3]. Two major trials, Dapagliflozin and Prevention of Adverse Outcomes in Heart Failure (DAPA-HF) and Empagliflozin Outcome Trial in Patients with Chronic Heart Failure and a Reduced Ejection Fraction (EMPEROR-Reduced), showed that inhibitors of sodium–glucose cotransporter 2 (SGLT2i) reduce the risk of hospitalization for heart failure (HF) regardless of the presence or absence of diabetes [4 5].
A recent study, the Empagliflozin Outcome Trial in Patients with Chronic Heart Failure with Preserved Ejection Fraction (EMPEROR preserved trial), demonstrated that empagliflozin reduced the CV death or hospitalization for HF, even in patients with HF preserved ejection fraction (HFpEF), regardless of the presence or absence of diabetes [6].

The SGLT2 is localized to the renal proximal convoluted tubules, acting to reabsorb the majority (≈90%) of the filtered glucose coupled with sodium. SGLT2 inhibition therefore results in glucosuria, and the ensuing osmotic diuresis may potentially be beneficial particularly for those with T2D and HF. Whether SGLT2 inhibitors cause significant natriuresis is less clear, but is important in the context of patients with HF who are likely to also be prescribed loop diuretics.[7 8 9].
Understanding the diuretic and natriuretic interplay between SGLT2 inhibitors such as empagliflozin and conventional loop diuretics is therefore critical for optimizing therapy in this high-risk population.There is a study approved by the East of Scotland Research Ethics Service (Regional Ethics Committee reference 16/ES/0137), RECEDE-CHF trial (SGLT2 Inhibition in Combination with Diuretics in Heart Failure) (NCT03226457) methodology have been published [10].

Sodium–glucose co-transporter 2 (SGLT2) inhibitor- Empagliflozin

Empagliflozin is a selective inhibitor of SGLT2, providing dose-dependent UGE increases in healthy volunteers, with up to 90 g of glucose excreted per day. It can be administered orally, and studies of people with renal or hepatic impairment indicated empagliflozin needed no dose adjustment based on pharmacokinetics [11]. Two members of the SGLT family are involved in glucose reabsorption in the kidney: SGLT2 is a high-capacity, low-affinity transporter, expressed in the early convoluted segment of the proximal tubule, and has traditionally been thought to be responsible for nearly 90% of the active renal glucose reabsorption [12-13]. while SGLT1, a high-affinity, low-capacity transporter, expressed in the distal segment of the proximal tubule, reabsorbs the remaining 10% [14-15]. Empagliflozin has SGLT2-dependent effects on the kidney (modulating fluid balance, metabolism, and hemodynamic), but early preclinical research has also demonstrated off-target effects of SGLT2i on cells largely devoid of SGLT2 [16]. Mechanisms explaining the cardiac effects of SGLT2i are likely multiple and largely independent of the SGLT2 expression in the heart. The activation of multiple signalling pathways rather than the direct binding of a hypothetical cardiac receptor, may better explain the multiple effects of these drugs on the heart [17].

Property	**Empagliflozin**
Relative potency	High (selective for SGLT2 > SGLT1, ~2500-fold)
Bioavailability (%)	~78%
Oral: Intravenous dosing	Oral only (no IV formulation)
Time to onset (hr.)	1 – 2
Oral peak serum concentration (hr.)	1.5
Absorption affected by food	No significant effect
Average half-life (hr.)	12 – 13
Duration of effect (hr.)	~24 (once-daily dosing)
Additional benefits	Cardioprotective, Reno protective

Table 2: Pharmacological Properties of (SGLT2) inhibitor.

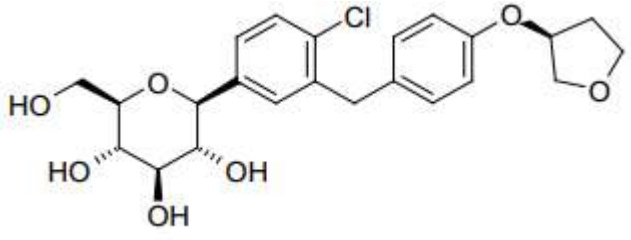

Figure 1: Empagliflozin Structure

Loop Diuretics – Furosemide

Congestion and fluid retention are the hallmarks of decompensated heart failure (HF) and the major reason for the hospitalization of patients with heart failure [18]. The loop diuretics possess a unique pharmacology and pharmacokinetics that lay the ground for different strategies to increase diuretic efficiency. However, many of these approaches have not been evaluated in randomized clinical trials (RCTs). In recent years, a stepped and protocolized diuretics dosing has been suggested to have superior benefits over an individual clinician-based strategy [19]. A literature review was conducted through a search of MEDLINE and EMBASE databases using the following keywords: "heart failure; diuretics; clinical trial; review; meta-analysis; guideline" until 30 June 2024. Excluded were studies with only abstracts available or studies that focused on diuretics use among patients with hypertension only [20].

Loop diuretics inhibit the $Na^+/2Cl^-/K^+$ cotransporter in the thick ascending loop of Henle, resulting in increased excretion of urinary sodium and chloride and subsequent diuresis. Bumetanide and torsemide have consistent bio-availabilities of 80–100% compared with the wide range of 10–100% for furosemide [21-22]. (Table 1)

Property	Furosemide	Torsemide	Bumetanide
Relative potency	1x	2x	40x
Bioavailability (%)	10 – 100	80 – 100	80 – 100
Oral:Intravenous dosing	2:1	1:1	1:1
Time to onset (min)	60	60	30 – 60
Oral peak serum concentration (hr)	1	1	1 – 2
Absorption affected by food	Yes	No	Yes
Average half-life (hr)	2	3.5	1 – 1.5
Duration of effect (hr)	6 – 8	6 – 16	4 – 6
Decreased kaliuresis	No	Yes	No

Abbreviations: *hr: hour; min: minute*

Table 1: Pharmacological properties of Furosemide

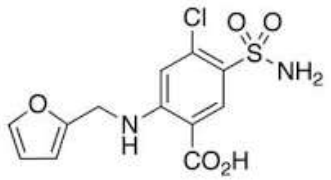

Figure 2: Furosemide structure.

Empagliflozin & Furosemide in combination

A variety of methods have been used to estimate and validate Empagliflozin both alone and in combination [23]. The most frequent separation technique in HPLC is reversed-phase chromatography. The reversed-phase method's versatility and ability to handle compounds with varying polarities and molecular masses contribute to its popularity. For the determination of furosemide in the bulk and its tablet dosage form, a simple, rapid, precise, and accurate UVspectrophotometric method was developed and validated. The maximum absorbance of furosemide with this method can be observed at

229 nm [24]. There are several methods for estimation and validation single-fixed dose been published and theoretically proven, but there is no proven or validated analytical method for combination of empagliflozin and furosemide.

CONCLUSION

Heart failure remains a major global health challenge, and despite the established role of loop diuretics in relieving congestion, their limitations highlight the need for adjunctive therapies. Sodium–glucose co-transporter 2 inhibitors, particularly empagliflozin, have demonstrated significant benefits in reducing hospitalizations for both reduced and preserved ejection fraction heart failure, independent of diabetes status. Their unique mechanisms, including osmotic diuresis and pleiotropic cardiovascular effects, complement the natriuretic action of loop diuretics such as furosemide. Although individual analytical methods for empagliflozin and furosemide are available, there is currently no validated method for their simultaneous estimation in a fixed-dose combination. Addressing this analytical gap is essential for developing combination therapies that may optimize treatment outcomes in heart failure patients.

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