



Review on bioanalytical and analytical method development of two potassium-sparing diuretics, namely eplerenone and spironolactone
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ABSTRACT

Eplerenone (EPL) and spironolactone (SPR) belong to the class of potassium-sparing diuretics. Both drugs are prescribed for the treatment of hypertension and heart failure. Spironolactone is structurally like progesterone and binds to progesterone, androgen and mineralocorticoid receptors. Eplerenone is a selective mineralocorticoid receptor antagonist, so it lacks the anti-androgenic side effects of spironolactone. Using a thorough computer assisted literature survey; this review article touches upon the reported analytical methods for the quantification of both drugs in raw materials, pharmaceutical dosage forms and biological fluids. Bioanalytical methods are widely used for quantitative estimation of drugs and their metabolites in biological matrices. Various analytical methods such as spectrophotometry, spectrofluorimetry, HPLC, TLC, and UPLC have been used in laboratories for the quantitative analysis of these drugs in biological samples and pharmaceutical preparations. Therefore, the aim of this review is to give a summary of the current analytical methods used for the assay of both drugs. The review represents a guide for the QC labs and the bioequivalence centers to analyze the studied drugs.

Keywords: *Eplerenone, Spironolactone, Diuretics, Analytical review*

1. Introduction

Spironolactone (SPR) is a steroid with a structure that is similar to natural adrenocortical hormone aldosterone, so it acts as a competitive antagonist of aldosterone. SPR is a potassium-sparing diuretic, reducing potassium excretion and increasing sodium and water excretion. It is reported to have a relatively slow onset of action. SPR is used in

the management of heart failure. In the treatment of oedema, SPR is usually given in an initial oral dose of 100 mg daily and in some patients may require doses of up to 400 mg daily. Potassium supplements should not be given with spironolactone. SPR chemically is: (2R)-7 α -(Acetylsulfanyl)-3',4'-dihydro-5'H-spiro[androst-4-ene-17,2'-furan]-3,5'-dione (**Fig. 1**). Eplerenone (EPL) is an aldosterone antagonist with properties similar to those of SPR but with a higher selectivity for the aldosterone receptor. It is given orally in the management of hypertension and heart failure. In the management of hypertension, EPL may be given alone or with other antihypertensives. It is given in an initial dose of 50 mg daily, increasing if necessary to 50 mg twice daily. EPL chemically is: 9,11 α -Epoxy-7 α -(methoxycarbonyl)-3-oxo-17 α -pregn-4-ene-21,17-carbolactone (**Fig. 2**) (Brayfield, A., 2014). It is necessary to observe their plasma levels mainly in patients who suffer from kidney problems as both drugs can cause serious complications. This review article gives broad information to the readers about the various analytical methods available for the determination of SPR and EPL in their pharmaceutical dosage forms and biological fluids. The current relevant analytical trends and prospective analytical methods for SPR and EPL have been presented and discussed. SPR and EPL have been analyzed by various methods which have been described in different literatures.

2. Analytical review of SPR

According to literature review, many chromatographic and spectrophotometric methods for the determination, quantification, stability indication and separation of SPR in formulation, bulk, and biological fluids have been developed. HPLC, HPTLC, UV-Visible spectroscopic techniques, LC – Hyphenated system, electrophoresis, spectrofluorimetry and electrochemical methods were among the techniques used in the experiments.

2.1. Spectroscopic methods

2.1.1. Spectrophotometric methods

Many spectrophotometric methods were reported for the determination of SPR either in raw material or pharmaceutical preparations. A summary of the reported spectrophotometric methods is shown in (**Table 1**).

2.1.2. Spectrofluorimetric method

A spectrofluorimetric method depends on measuring the quenching of fluorescence of nitrogen doped carbon quantum dots by SPR. The method was linear over the range of 0.5 to 6.0 $\mu\text{g/mL}$ with LOQ of 0.262 $\mu\text{g/mL}$. The method was further extended for determination of the drug in its pharmaceutical tablets and spiked human plasma (Abo Zaid et al., 2023).

Another spectrofluorimetric method depends on measuring the quenching of the fluorescence of CdSe quantum dots (QDs) by spironolactone. In the optimum conditions, SPR concentration versus quantum dot fluorescence gave a linear response in the concentration range of 2.5 and 700 $\mu\text{g/mL}$ with LOD of 0.2 $\mu\text{g/mL}$ (Liang et al., 2006).

Another spectrofluorimetric method for the simultaneous determination of canrenone and SPR in urine was reported. The method was based on the different rates at which the two analytes react with hot sulfuric acid to form a trienone. The kinetic spectrofluorimetric data were processed by partial least-squares regression. The effects of sulfuric acid concentration and temperature on the system were also evaluated and the optimum values for carrying out the reaction were 50% and 50 degrees C, respectively. The method was checked by analyzing urine samples that contained both diuretics (Hernández et al., 2000)

2.2. Separation techniques

2.2.1. Chromatographic methods

2.2.1.1. High performance liquid Chromatography (HPLC)

Various HPLC methods were applied for determination of SPR and can be summarized as follows (**Table 2**).

2.2.1.2. Ultra performance liquid chromatography (UPLC)

Ultra-performance liquid chromatographic (UPLC) method was developed for quantitative determination of furosemide and SPR in bulk sample and pharmaceutical dosage form in the presence of degradation products. Isocratic separation was carried out on C₁₈ column using a mobile phase containing a mixture of sodium dihydrogen phosphate monohydrate and acetonitrile (57:43) (V/V) at flow rate of 0.25 mL/min using UV detection at 235 nm (Bhatia & Dahivelkar, 2013).

2.2.2. Capillary electrophoresis (CE)

Capillary zone electrophoresis method was reported for the quality control of spironolactone in three different formulation types and a rapid simultaneous determination of the content of SPR and canrenone in urine samples. After optimization of separation conditions, the electrolyte solution was the pH 5.5, 20 mM phosphate buffer containing 4.5 g/L sulfated- β -cyclodextrin, 15 kV of electric field across the capillary applied at 25°C. A diode array detector was used, and the detection wavelength was 260 nm (Li et al., 2016).

2.3. Electrochemical methods

A stripping voltametric method was reported for determination of SPR. This method is based on the adsorptive accumulation of the drug at a hanging mercury drop electrode and then a negative sweep was initiated, which yield a cathodic peak at -1000 mV versus Ag/AgCl reference electrode. The monitored adsorptive current was directly proportional to the concentration of SPR and it shows a linear response in the range from 1×10^{-8} to 2.5×10^{-7} mol/L (Al-Ghamdi et al., 2008).

Another voltametric technique was reported. Where the working electrode amalgam film silver-based electrode was applied. The linear response of SPR signal on mercury film electrode was obtained in the range of concentration from $15 \cdot 10^{-9}$ to $3.0 \cdot 10^{-6}$ mol L⁻¹ and the obtained repeatability measured as RSD was very good, with the value of 2.0%. The proposed method was successfully applied in commercially available pharmaceutical formulations containing known amount of SPR (Smajdor, Piech & Bator, 2018).

3. Analytical review of EPL

According to literature survey, many chromatographic and spectrophotometric methods for the determination, quantification and separation of EPL in formulation, bulk, and biological fluids have been developed. HPLC, HPTLC, UV-Visible spectroscopic techniques, LC – Hyphenated system, Electrophoresis, and Spectrofluorimetry were among the techniques used in the experiments.

3.1. Spectroscopic methods

3.1.1. Spectrophotometric methods

Many spectrophotometric methods were reported for the determination of EPL either in raw material or pharmaceutical preparations. A summary of the reported spectrophotometric methods is shown in (Table 3).

3.1.2. Spectrofluorimetric methods

A spectrofluorimetric method depends on measuring the quenching of fluorescence of nitrogen doped carbon quantum dots by EPL. The method was linear over the range of 0.5 to 5.0 $\mu\text{g/mL}$ with LOQ of 0.383 $\mu\text{g/mL}$. The method was further extended for determination of the drug in its pharmaceutical tablets and spiked human plasma (Abo Zaid et al., 2023).

Another spectrofluorimetric method depends on measuring the quenching of the fluorescence of S, N-CDs by EPL. The decreased intensity of S, N-CDs fluorescence was proportional to EPL in the 0.2–3.0 μM range. The limit of detection and quantitation were 0.05 and 0.15 μM , respectively. The assay of EPL was successfully carried out in drug formulations and in spiked human serum samples (Belal et al., 2020).

3.2. Chromatographic methods

3.2.1. High performance liquid Chromatography (HPLC)

Various HPLC methods were applied for determination of EPL and can be summarized as follows (Table 4).

3.2.2. Thin layer chromatography (TLC)

A thin-layer chromatography /densitometry method has been reported for determination of EPL in bulk drug and in tablet formulation. Aluminium foil TLC plates precoated with silica gel 60F₂₅₄ were used as the stationary phase and ethyl acetate: toluene: triethylamine 6:4:0.4 (v/v) as mobile phase. A compact band (R_f 0.45 \pm 0.02) was obtained for EPL. Densitometric analysis was performed in the absorbance mode at 241 nm (Mahajan, Keskar, Shah, 2011).

3.2.3. High performance thin layer chromatography (HPTLC)

High-performance thin-layer chromatographic method (HPTLC) has been reported for simultaneous estimation of EPL and toseamide. A mixture of ethyl acetate -methanol- chloroform (8:2:1, v/v/v) was applied as mobile phase.

EPL was well resolved and scanned at 242 nm (at hR_F 77). The method was found to be linear between 50–600 ng/band for EPL (Younis et al., 2020).

4- Conclusion

SPR and EPL are potassium-sparing diuretics used for the treatment of hypertension. The analytical profile of each drug highlights various analytical methods for the determination of both drugs in raw materials, formulations and biological fluids. The review would assist the analytical chemist in understanding the essential solvents and their combinations for instruments that are available in the analytical laboratory.

Disclosure

The authors report no conflicts of interest in this work.

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Figures

Fig. 1: Chemical structure of SPR.

Fig. 2: Chemical Structure of EPL.

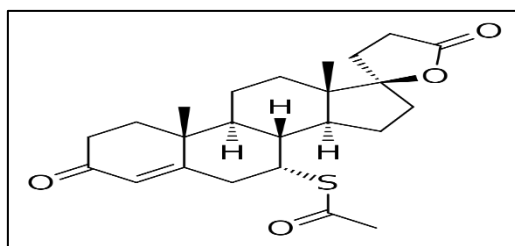


Fig. 1

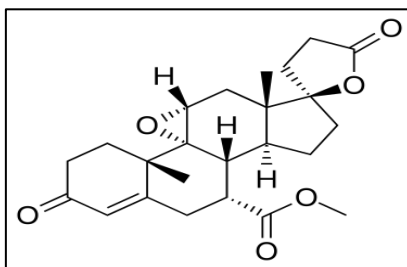


Fig. 2

Tables

Table 1: Reported spectrophotometric methods for the determination of SPR.

Matrix	Method	λ_{\max} (nm)	Ref.
SPR and Torsemide in tablets	First Derivative	Zero crossing point at 315 nm	(Golher et al., 2010)
	Absorbance ratio method	Isoseptic point at 238 nm	
SPR and Hydrochlorothiazide in bulk and pharmaceutical preparations	Isoseptic point	232.4 nm and 257.6 nm	(Hegazy et al., 2010)
	Ratio Subtraction method	243.8 nm	
	Chemometric methods (PLS & GA-PLS)		
SPR and furosemide in tablets	Principal component regression	235 nm	(Israt et al. 2016)
SPR in bulk and tablets	First Derivative (1D)	226 nm	(Prajapati et al., 2016)
	Second Derivative (2D)	262 nm	
SPR and Hydrochlorothiazide in tablets	Absorbance ratio method	Isoseptic point at 260 nm	(Tekerek et al., 2008)

Table 2: Reported High Performance Liquid Chromatographic methods for the determination of SPR.

Application (s)	Mobile phase	Column	Detection	Ref.
SPR and Losartan in tablets	Methanol: water (70:30)	C ₁₈	UV detection at 240 nm	(Chaure et al., 2019)
SPR in human plasma	0.1 % formic acid in water: methanol (30: 70, v/v)	C ₁₈	MS/MS	(Lee et al., 2015)
SPR and furosemide in tablets	Acetonitrile: ammonium acetate buffer (50:50, v/v) at pH 3.9	C ₈	UV detection at 254 nm	(Ram et al., 2012)
SPR and its metabolites in paediatric plasma samples	Methanol: water (60:40, v/v)	C ₁₈	UV detection at 238 nm	(Sandall et al., 2006)
SPR and Losartan in tablets	Acetonitrile: 0.025 M KH ₂ PO ₄ (60:40, v/v) adjusted to pH 3.49 with ortho - phosphoric acid	C ₈	UV detection at 235 nm	(Sebaiy et al., 2020)
SPR, furosemide and canrenone in human plasma samples	(3:7 aqueous 0.1% formic acid and methanol, v/v) at a flow rate of 0.8 mL/min	C ₁₈	MS/MS	(Sora et al., 2010)
SPR and canrenone in human plasma	Mobile phase of 58 % methanol, 42 % 10 mmol dm ⁻³ ammonim acetate in water	C ₁₈	MS/MS	(Vlase et al., 2011)
SPR and furosemide in combined tablets and human plasma	A mixture of 0.1 M sodium dodecyl sulphate (SDS), 0.3% triethylamine (TEA), 10 % n-propanol all prepared in 0.02 M orthophosphoric acid at pH 4.0	C ₁₈	UV detection at 238 nm	(Walash et al., 2012)

Table 3: Reported spectrophotometric methods for the determination of EPL.

Matrix	Method	λ_{\max} (nm)	Ref.
EPL in bulk and tablets	Measuring absorbance in methanol-water (80:20)	242.5 nm	(Banode, Khedekar & Tarte, 2011)
EPL in bulk and tablets	Reaction of keto group of eplerenone with 2, 4-dinitrophenyl hydrazine	430 nm	(Ganure et al., 2011)
EPL and Torsemide in bulk and tablets	Absorbance ratio (Q- analysis method)	239.80 nm (λ max of Eplerenone) and 260 nm (Iso absorptive point).	(Hinge, Petal &Patel, 2019)
EPL in tablets	Ion association complex with Bromo Thymol Blue	410 nm	(Khan et al., 2012)
	Bromo Cresol Green	414 nm	
	Bromo Phenol Blue	425 nm	
EPL and Torsemide in tablets	Chemometrics a (multivariate) method which includes classical least square (CLS) and inverse least square (ILS)	Absorbance measured in the spectral region of 240-300 nm	(Madhuri, Patel, 2021)
EPL and Torsemide in tablets	Q- Absorbance Ratio method	Iso-absorptive point at 262 nm and 244 nm (λ max of Eplerenone)	(Tathe et al., 2021)
EPL and Torsemide in tablets	Second derivative ratio	The values of DD^2 amplitudes were measured at 244–260 nm (peak-to-peak) and 214 nm for EPL	(Younis et al., 2020)
	Ratio subtraction (RS) method	EPL solutions were recorded at 242 nm.	

Table 4: Reported High Performance Liquid Chromatographic methods for the determination of EPL.

Application (s)	Mobile phase	Column	Detection	Ref.
EPL in tablets	A mixture of 10 mM tetra butyl ammonium hydrogen sulfate and methanol (30: 70, v/v)	C ₁₈	UV detection at 242 nm	(Annapurna, Madhuri, Valli, 2018)
EPL in bulk and tablets	Acetonitrile and Phosphoric acid 0.1% (50:50, v/v).	C ₁₈	UV detection at 241 nm	(Asif et al., 2017)
EPL and torsemide in tablet	Mobile phase (acetonitrile, water and methanol in the ratio of 50: 30: 20 v/v/v respectively)	C ₁₈	UV detection at 223 nm	(Hinge et al., 2022)
EPL and Torsemide in tablets	Acetonitrile: Methanol: water (30:50:20 % v/v/v)	C ₁₈	UV detection at 268 nm	(Patel et al., 2016)
EPL and its metabolite in human plasma	A mobile phase of acetonitrile/water (40:60, v/v) containing 10 mM ammonium acetate 8 (pH 7.4)	C ₈	MS/MS	(Zhang et al., 2003)