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Compatibility determination of potassium orotate with spironolactone by high-performance liquid chromatography

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Abstract

Background: Compatibility determination between active pharmaceutical ingredients (APIs) in fixed-dose combinations is an indispensable step in the elaboration. High-performance liquid chromatography provides information on possible interactions between APIs and their related interaction products. The purpose of the present study was to investigate the compatibility of potassium orotate in combination with spironolactone by a HPLC method.
Material and methods: The detection was carried out using Liquid Chromatograph Agilent 1100 with UV-VIS detector and a RP-18 reversed column (250*4 mm, 5 µm), mobile phase of acetonitrile: phosphate buffer solution (pH=4.0) with the ratio 1:49 and 1:1, at flow rate 1 and 1.5 mL/min, injection volume 20 µL; potassium orotate and spironolactone substances were provided by Sigma Aldrich, USA.
Results: Due to the developed method both separation and simultaneous qualitative and quantitative determination of APIs in the mechanical mixture were carried out. Spironolactone: retention time 6.9 min, concentration 98.1% (±0.21); potassium orotate: retention time 3.06 min, concentration 91.67% (±0.15). There were just well-separated symmetrical peaks of APIs and no additional peak in the chromatograms.
Conclusions: There is compatibility between APIs. Further studies will be performed by other methods (DSC, FT-IF Spectrometry) to confirm the obtained result.
Key words: HPLC, combination, potassium orotate, spironolactone.

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Introduction

Nowadays, the number of new fixed-dose combinations (FDCs) is rising significantly. FDC is a medicine that includes two or more active pharmaceutical ingredients (APIs) combined in a single dosage form. Due to this, the FDCs have several advantages over monocomponent medicine, such as potentiating the therapeutic efficacy, reducing the incidence of adverse effect of medicines, having pharmacokinetic advantage, better compliance by reducing the pill burden, reducing dose of individual medicines, decreasing development of resistance, ensuring cause treatment. No less important they are cheaper than individual medicine because of reduced cost from packaging to distribution. Nowadays, monotherapy is an unsuccessful treatment of many cases, particularly chronic diseases, such as hypertension, diabetes, immunodeficiency virus (HIV), tuberculosis as well as hypopotassemia [1, 2, 3].

Hypopotassemia is serum potassium level less than 3.6 mEq per L (3.6 mmol per L). It is a common electrolyte disorder, which occurs in up to 21% of hospitalized patients and 2% to 3% of outpatients [4, 5]. Hypopotassemia occurs in less than 1% of healthy individuals, but is present in up to 20% of hospitalized patients, 40% of patients taking diuretics, and 17% of patients with cardiovascular conditions [6, 7]. Furthermore, as many as 20% of hospitalized patients are found to have hypopotassemia but only in 4–5% this is clinically significant [8]. The high prevalence of hypopotassemia among patients with COVID-19 suggests the presence of disordered renin-angiotensin system activity, which increases as a result of reduced counter activity of angiotensin-converting enzyme 2, which is bound by severe acute respiratory syndrome coronavirus 2 [9].

Potassium (K^+) plays a key role in maintaining normal cell function [10, 11]. K^+ is the main intracellular cation and almost all cells have the pump called 'Na^+-K^+-ATPase', which pumps sodium (Na^+) out of the cell and K^+ into the cell leading to a K^+ gradient across the cell membrane (K^+ in > K^+ out), which is partially responsible for maintaining the potential difference across membrane [4, 11]. Many cell functions rely on this potential difference, particularly in excitable tissues, such as nerve and muscle. Two percent of K^+ exist in the extracellular fluid (ECF) at a concentration of only 4mEq/L [10, 12]. Potassium is the third essential ele-

ment in the human body after calcium and phosphorus and is the most abundant cation in the intracellular compartment, which facilitates nerve impulse conduction and the contraction of skeletal and smooth muscles, including the heart. It controls the formation and storage of glycogen and prevents the calcium loss through the urine. If there is a potassium lack, the lungs cannot remove the carbon dioxide and the kidneys cannot concentrate urine, resulting in excessive urination [13].

Hypopotassemia is frequently asymptomatic finding identified only on routine electrolyte screening. Clinical symptoms and signs of hypopotassemia depend on the rate of onset and severity. It can be associated with symptoms ranging from the confusion, disorientation, weakness and discomfort of muscles to the arrhythmias, muscle cramps (paralysis), respiratory failure and sudden death [8, 14]. Hypopotassemia may be iatrogenically caused by other medicines, such as furosemide, insulin, gentamicin, theophylline and salbutamol. Appropriately 80% of patients who are receiving diuretics (thiazide, loop) become hypopotassemic, while many of patients with hypopotassemia could also have an associated systemic disease [12, 13, 15]. Therefore with the application of loop diuretics and digoxin, hypopotassemia has become a frequent and feared side effect of treatment.

The correction of hypopotassemia depends on its severity: mild (plasma potassium 3.1-3.5mmol/L), moderate (plasma potassium 2.5-3.1mmol/L) and severe (plasma potassium <2.5mmol/L). Urgent treatment is warranted for patients with potassium levels less than 2.5mEq/L by intravenous potassium chloride at 10-20 mEq/hour applying ECG monitoring and continuously careful estimation of serum potassium levels [12]. Mild and moderate hypopotassemia may be corrected by using an oral potassium supplementation (potassium chloride, potassium orotate, potassium aspartate) [16]. The most important is to correct underlying causes, for example, thiazide and loop diuretics can be replaced with the potassium-sparing diuretics, such as spironolactone that leads to minimize potassium loss [17]. It is well-known that hypopotassemia can be induced by hypomagnesemia, therefore combination of potassium and magnesium aspartate provides potassium and magnesium correction, which resolves the cause of the imbalance [14, 18].

To gain the maximum benefit from treatment, we need to use fixed-dose combinations that improve the potassium supplementation and ensure etiological treatment in hypopotassemia. Unfortunately, there is a deficiency of a combined local pharmaceutical product on the pharmaceutical market of the Republic of Moldova. Therefore, the pharmaceutical product, which consists of potassium orotate, potassium and magnesium aspartate, spironolactone, is in the process of development at the Scientific Center of Medicine. Due to this complex composition this new pharmaceutical product can be applied not only to eliminate the symptoms of hypopotassemia (weakness, cramps), but also to ensure the causal treatment. Resulting from the fact, that combination of potassium and magnesium aspartate provides potassium and magnesium correction, because hypomagnesemia is linked to potassium imbalance. Hypopotassemia in individuals with high blood pressure, who require taking thiazide diuretics, may be improved by replacement or combination of it with a potassium-sparing diuretic (spironolactone). Very often potassium is used as an orotic acid salt that is a non-steroidal anabolic agent, which helps to normalize the external electrolyte balance of potassium through the stimulation of metabolic processes. Thus, due to these active substances combined in the single dosage form for oral administration, it can produce better effect in the treatment of hypopotassemia, than any of them taken separately. Therefore, it is now in the active process of development at the Scientific Center of Medicine [18, 20].

Definitely, various regulatory authorities, such as the Food and Drug Administration (FDA) and International Conference on Harmonization (ICH) require the pre-formulation studies on each new FDC. Pre-formulation testing is an important step in the development of new products or the reformulation of existing ones. It includes the chemical characterization of the medicine and analytical compatibility/stability tests. In the pharmaceutical industry, the active pharmaceutical ingredient is subjected to pre-formulation studies, which provide the necessary information for the development of a stable medicine formulation with adequate bioavailability [21]. Thus, it is designed to find out if the APIs have the potential to interact with each other or between APIs and excipient pharmaceutical ingredients (EPIs) [22, 23].

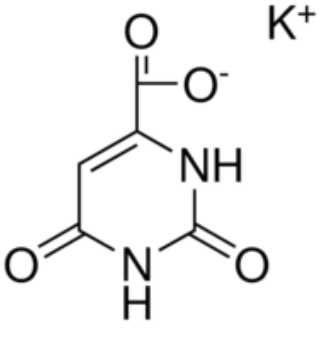

Fig. 1. Chemical structure of potassium orotate

Incompatibility is an undesirable chemical or physical reaction between the API and EPIs or between two or more APIs that could reduce their effectiveness or result in toxicity, increasing of minor or serious unexpected side effects. Physical and chemical interactions between APIs can affect the chemical nature, the stability and bioavailability of medicine products and consequently, their therapeutic efficacy and safety [24, 25].

Evaluation of possible incompatibilities between the APIs is an important part of the preformulation phase during the development of a dosage form. Successful compatibility studies require a good experimental design that furnishes the required information with the minimum of

experimental effort. Definitely, not all APIs interactions are so bad, but major of them are life-threatening, therefore it is so necessary to determine them at the beginning of the medicine development phase by applying sensible and modern methods. Despite the importance of compatibility test between APIs, there is no universal protocol for this study. It is a key step in determining the success of medicine development [26].

To investigate the compatibility of the components of a formulation, techniques, such as X-ray diffraction, Fourier-transform infrared spectroscopy (FT-IR spectroscopy), high-performance liquid chromatography (HPLC) and thermal analysis (especially differential scanning calorimetry – DSC) are used [27-30]. Although DSC is a fast and reliable technique and generally regarded as one of the methods of first choice in assessing pharmaceutical compatibility study, the evaluation of the curves is often difficult [31]. Therefore, this study was based on HPLC method, because it is easier than DSC and carries out qualitative and quantitative determinations of APIs and their related interaction products. Therefore, this method compares favorably with that commonly employed DSC and the values obtained by HPLC are of special importance.

The principal purpose of this study was to investigate the possible compatibility between the potassium orotate and spironolactone in the mixture by comparing the HPLC-obtained chromatograms. Consequently, this study serves to give information on a medicine's interaction, using HPLC method by providing not only qualitative but also quantitative results for pure APIs and their related interaction products. Analyses of HPLC chromatograms obtained on pure APIs and combinations of them made it possible to determine the compatibility between APIs by comparing the following values: the number, shape and size of peaks. Due to the chemical structure of potassium and magnesium aspartate and specifically to the lack of chromatophore groups they couldn't be investigated by HPLC. Thus, HPLC method was applied for spironolactone and potassium orotate.

Potassium orotate is chemical potassium 2.4-dioxo-1H-pyrimidine-6-carboxylate (potassium Uracil-6-carboxylate) (fig. 1). Its molecular formula is $C_5H_3KN_2O_4$ and its molecular weight is 194.19 g/mol. Potassium orotate is a bioavailable form of potassium (orotic acid helps it (K+) pass easily through cell membranes), that supports the nervous system, kidneys, cardiovascular health, bone strength, and encourages a positive response to stress and anxiety. Definitely, orotic acid is an intermediate in the pyrimidine biosynthesis, which is required for DNA and RNA synthesis.

Spironolactone is a synthetic 17-spironolactone corticosteroid with potassium-sparing diuretic, antihypertensive, and antiandrogen activities (fig. 2). Spironolactone competitively inhibits adrenocortical hormone aldosterone activity in the distal renal tubules (it actually works on aldosterone-dependent sodium- potassium exchange channels). This increases the excretion of water and sodium, while decreasing the excretion of potassium. The increased excretion of water leads to diuretic and also antihypertensive effects. Spironolactone has a fairly slow onset of action, taking several days to develop; similarly, the effect diminishes slowly.

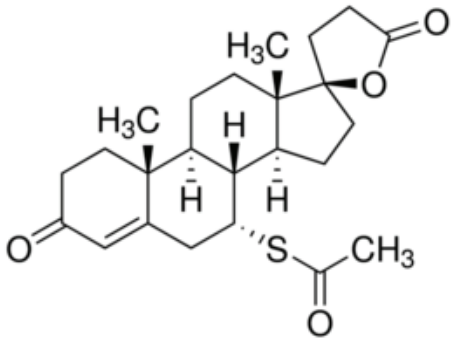

Fig. 2. Chemical structure of spironolactone

Material and methods

The laboratory analyses were performed in the Laboratory of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Graz, within the doctoral research mobility CIII-RO-0010-14-1920 – "Teaching and Learning Bioanalysis" under the leadership of professor Martin Schmid.

Materials

The HPLC-grade potassium orotate, HPLC-grade spironolactone, HPLC-grade acetonitrile (ACN) and analytical-grade potassium phosphate monobasic were provided by Sigma Aldrich (USA). HPLC-grade water was obtained from Fisher Chemical (Belgium).

Instrumentation and chromatographic conditions

The chromatographic analyses were performed by Liquid Chromatograph Agilent 1100 equipped with autosampler. The study was made on a RP-18 reversed column (250mm long by 4 mm internal diameter, particle size 5μm) and by an isocratic elution method.-

There are 2 elution techniques of pumping mobile phase through a column: isocratic and gradient methods. In the isocratic method, the composition of the mobile phase remains constant, whereas in the gradient method the composition changes during the separation process. The isocratic method is the simplest technique and should be the first choice when developing a separation. Therefore, isocratic method of compatibility determination was selected to determine compatibility of these studied 2 APIs in the mixture.

In order to select a proper mobile phase for the good separation and quantitative determination of potassium orotate and spironolactone simultaneously, the chromatographic conditions were selected after testing different parameters, such as diluents, buffer, buffer concentration, organic solvent for mobile phase, mobile phase composition, flow rate and temperature. Mobile phase selection was based on peak parameters (symmetry, tailing), run time, therefore two mobile phases were prepared.

Preparation of mobile phase N1: 2.72 g of potassium dihydrogen phosphate were weighed by balance (RAD-

WAG) and transferred to 1000 mL volumetric flask and dissolved by HPLC-grade water then completed to the mark by HPLC-grade water. The solution was adjusted to pH 4.0 with 2M orthophosphoric acid. HPLC-grade acetonitrile and obtained phosphate buffer solution 0.02M were mixed with (1:49) ratio. The mobile phase was degassed in an ultrasonic bath (GT SONIC Professional Ultrasonic Cleaner, China).

Preparation of mobile phase N2: 6.8 g of potassium dihydrogen phosphate were weighed by balance (RAD-WAG) and transferred to 1000 mL volumetric flask and dissolved by HPLC-grade water then completed to the mark by HPLC-grade water. The solution was adjusted to pH 4.0 with 2M orthophosphoric acid. HPLC-grade acetonitrile and obtained phosphate buffer solution 0.05M were mixed with (1:1) ratio. The mobile phase was degassed in the ultrasonic bath (GT SONIC Professional Ultrasonic Cleaner, China).

The study was performed on mobile phase N1 and at flow rate 1 mL/min, injection volume 20 µL, temperature 25°C. The detection was set at 254 nm for spironolactone and at 278 nm for potassium orotate using UV-visible absorption detector. Total run time was less than 20 min for each injection.

Preparation of standard and test solutions, made on mobile phase N1 (M/Ph. N1), in which potassium orotate is predominantly determined:

A standard and test potassium orotate solutions were prepared in a 10-mL volumetric flask by dissolving 15.00 mg of the substance to be examined and then diluting to volume with mobile phase. Then this obtained solution undergoes a sonication for 30 seconds and then it is completed to the mark by mobile phase. Dilute 1mL of this solution to 10.0mL with mobile phase. Place 1mL of the obtained solution in the 10-mL volumetric flask and make up to mark with mobile phase (the final solution contains 15 µg/mL). The solutions were scanned and found to have maximum absorption wavelength at 278 nm using mobile phase as blank.

A standard and test spironolactone solutions were prepared in the 10-mL volumetric flask by dissolving 2.40 mg of spironolactone and then diluting to volume with mobile phase. Then this obtained solution undergoes the sonication for 30 seconds and then it is completed to the mark by mobile phase. Place 1 mL of this solution in a 5-mL volumetric flask and made up to mark with mobile phase (the final solution contains 48 µg/mL). The solutions were scanned and weren't found to have maximum absorption wavelength at 240 nm using mobile phase as blank.

Test mixture preparation on M/Ph. N1: 2.40 mg of spironolactone and 15.00 mg of potassium orotate were weighed and mixed together with the ratio according to the amount of each APIs from the new fixed-dose combination, which is currently in progress. Whereupon, it was transferred into the 10-mL volumetric flask and then diluted to volume with mobile phase. The mixture was sonicated for a minimum of 30 seconds with intermittent shaking. Then the solution was brought back to room temperature and diluted to the mark with mobile phase. Dilute 1mL of this solution to 5 mL with mobile phase. Place 1mL of the obtained solution in the 10-mL volumetric flask and make up to mark with mobile phase.

As well as the study was performed on mobile phase N2 and at flow rate 1.5 mL/min, injection volume 20 µL, temperature 40°C. The detection was set at 254 nm for spironolactone and at 278 nm for potassium orotate using UV-visible absorption detector. Total run time was less than 10 min for each injection.

Preparation of standard and test solutions, made on mobile phase N2 (M/Ph. N2), in which spironolactone is predominantly determined:

A standard and test spironolactone solutions were prepared in the 10-mL volumetric flask by dissolving 2.40 mg of the substance to be examined and then diluted to volume with mobile phase. Then this obtained solution undergoes the sonication for 30 seconds and then it is completed to the mark by mobile phase. Dilute 1mL of this solution in the 5.0 mL with mobile phase. Place 1mL of the obtained solution in the 10-mL volumetric flask and make up to mark with mobile phase (the final solution contains 4.8µg/mL). The solutions were scanned and found to have maximum absorption wavelength at 240 nm using mobile phase as blank.

A standard and test potassium orotate solutions were prepared in a 10-mL volumetric flask by dissolving 15.00 mg of the substance to be examined and then diluting to volume with mobile phase. Then this obtained solution undergoes the sonication for 30 seconds and then it is completed to the mark by mobile phase. Dilute 1mL of this solution to the 5.0 mL with mobile phase. Place 1mL of the obtained solution in the 10-mL volumetric flask and make up to mark with mobile phase (the final solution contains 30µg/mL). The solutions were scanned and found to have maximum absorption wavelength at 278 nm using mobile phase as blank.

Test mixture preparation on M/Ph. N2: 2.40 mg of spironolactone and 15.0 mg of potassium orotate were weighed and mixed together with the ratio according to the amount of each API from the new fixed-dose combination. Whereupon, it was transferred into the 10-mL volumetric flask and then diluted to volume with mobile phase. The mixture was sonicated for a minimum of 30 seconds with intermittent shaking. Then the solution was brought back to room temperature and diluted to the mark with mobile phase. Dilute 1mL of this solution to 5 mL with mobile phase. Place 1mL of the obtained solution in the 10-mL volumetric flask and make up to mark with mobile phase.

Measurements were made on the standard and test solutions of spironolactone, standard and test solutions of potassium orotate and mixture of these APIs. Three replicated injections of each standard preparation and mixture were injected and analyzed. The peaks were detected at 254 nm for spironolactone and at 278 nm for potassium orotate and identified using reference standards of spironolactone and potassium orotate.

Three replicated injections of each standard and test preparation and mixture were injected and analyzed accord-

ing to the selected chromatographic conditions. The mean values of these three injections were used to evaluate retention time (t_R), area of peak (A), theoretical plate (N), resolution factor (Rs) and concentrations for APIs. The percent relative standard deviation (RSD, %), coefficient of variation (CV, %) values were used for evaluation of obtained values of spironolactone and potassium orotate separately and in the mixture. Concentrations of active substances in % were calculated according to the equation (1):

$$X\% = \frac{S_x * m_{st} * W_x * P_0}{S_{st} * m_x * W_{st}} * 100\%, \qquad (1)$$

where,

S_x – Average of area counts of principle peak obtained from the chromatograms of the test solution;

S_{st} -Average of area counts of principle peak obtained from the chromatograms of the standard solution;

m_x – mass of test substance, g;

m_{st} – mass of standard substance, g;

P_0 – Purity of reference standard (% w/w);

W_{st} *and* W_x – Dilution factor of standard and test solutions, respectively.

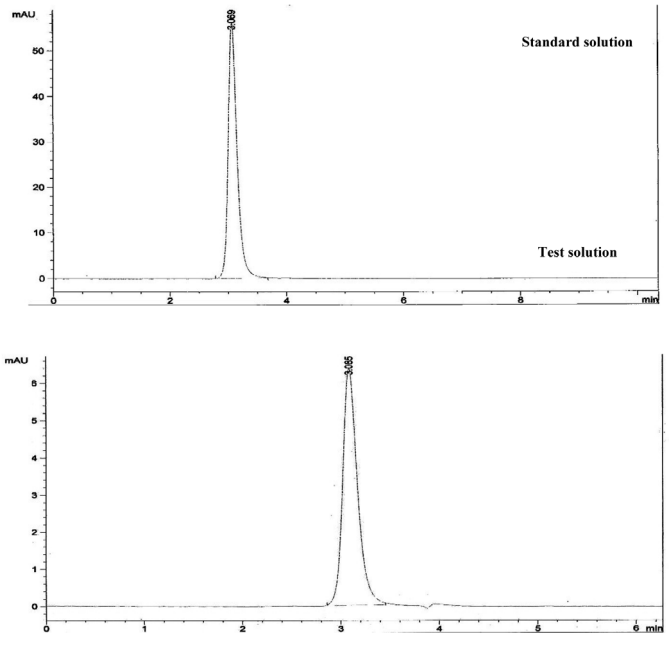

Fig. 1. Chromatograms of standard and test potassium orotate solutions at 278 nm on M/Ph No 1

Results and discussion

There were analyzed several methods to develop a suitable and effective HPLC method for both the compatibility determination of potassium orotate in the combination with spironolactone and quantitative determination of them.

Measurements were made on the pure substances, such as potassium orotate, spironolactone and mixture of them, using isocratic HPLC technique, carried out on the reversed phase chromatography and in the convenient chromatographic conditions. By accurate analyses of the obtained chromatograms for solutions of potassium orotate (fig. 1 and fig. 2), there were found the good shape of principal peak and its area, theoretical plate (N) and concentration, which are shown in table 1.

By accurate analyses of the obtained chromatograms for standard and test solutions of spironolactone (fig. 3), there were found the good shape of principal peak and its area, theoretical plate (N) and concentration, which are shown in table 2.

According to our preliminary data, it was found that the detection of potassium orotate is carried out at 278 nm with t_R 3.06 min and spironolactone at 240 nm with t_R 7.1min. Therefore, HPLC method for potassium orotate in combination with spironolactone was performed at different detection wavelengths, which are specific for each active substance.

By accurate analyses of the values obtained from the chromatograms for the mixture it was found that potassium orotate and spironolactone eluted out forming symmetrical peaks, which were well separated from each other, applying mobile phase No 2. There was no additional peak in chromatograms (fig. 4).

By comparing the obtained chromatograms, it was shown that the separation and simultaneous determination of two APIs on mobile phase N1 is difficult. Thus, it

Table 1

Evaluation data of potassium orotate chromatograms at 278 nm on M/Ph. N1 and No 2

	Retention time (t_R, min)		Theoretical plate (N)		Area of peak (A)		Concentration %	
	M/Ph. N1	M/Ph. N2	M/Ph. N1	M/Ph. N2	M/Ph. N1	M/Ph. N2	M/Ph. N1	M/Ph. N2
Potassium orotate	3.069	1.257	5976	2302	595.073	382.604	93.67	76.83
	3.083	1.267	6084	2312	595.553	385.362	93.38	76.69
	3.013	1.261	5709	2290	594.711	385.504	93.58	76.87
Mean. $\overline{XX}$	3.06	1.26	5923	2301	595.11	384.49	93.55	76.79
RSD. %	0.04	0.005	192.85	11	0.422	1.635	0.15	0.09
CV. %	1.21	0.40	3.26	0.47	0.07	0.43	0.16	0.12

Table 2

Evaluation data of spironolactone chromatograms on M/Ph N2

M/Ph N2	Retention time (t_R. min)	Theoretical plate (N)	Area of peak (A)	Concentration %
Spironolactone	7.019	16023	167.299	99.14
	7.147	15263	175.151	99.26
	7.148	16439	168.272	98.86
Mean. $\overline{XX}$	7.10	15908	170.24	99.09
RSD. %	0.074	596	4.28	0.20
CV.%	1.04	3.75	2.51	0.20

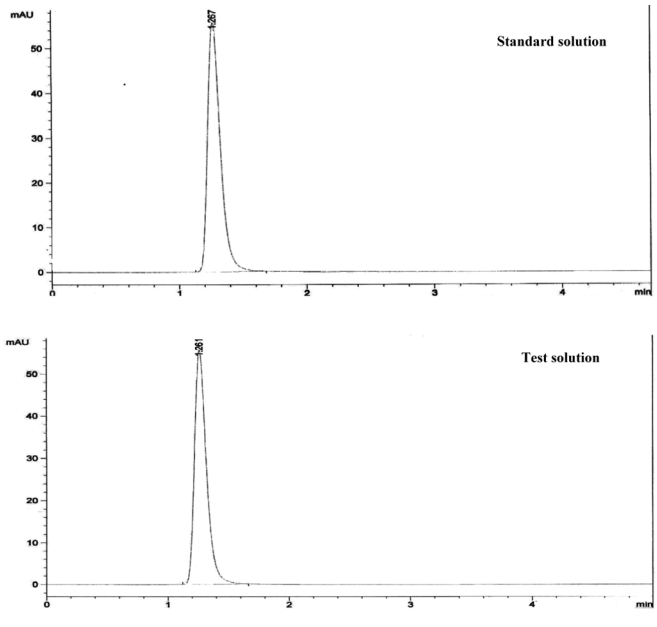

Fig. 2. Chromatograms of standard and test potassium orotate solutions at 278 nm on M/Ph No 2

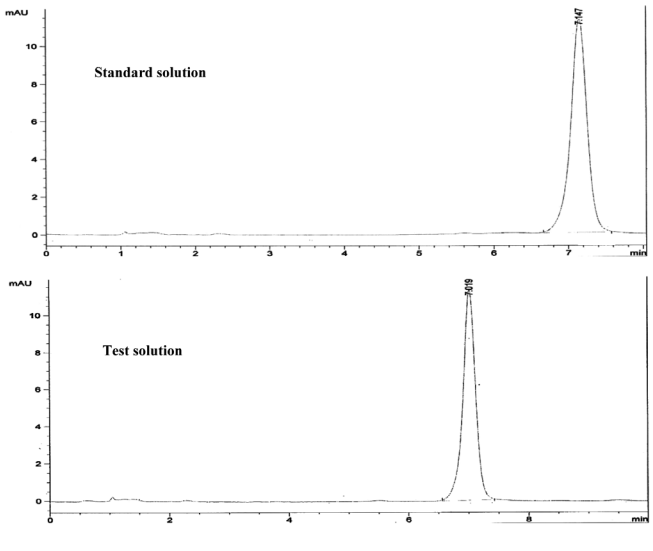

Fig. 3. Chromatograms of spironolactone standard and test solutions at 240 nm on M/Ph No 2

was concluded that the second mobile phase provided the good separation of potassium orotate and spironolactone.

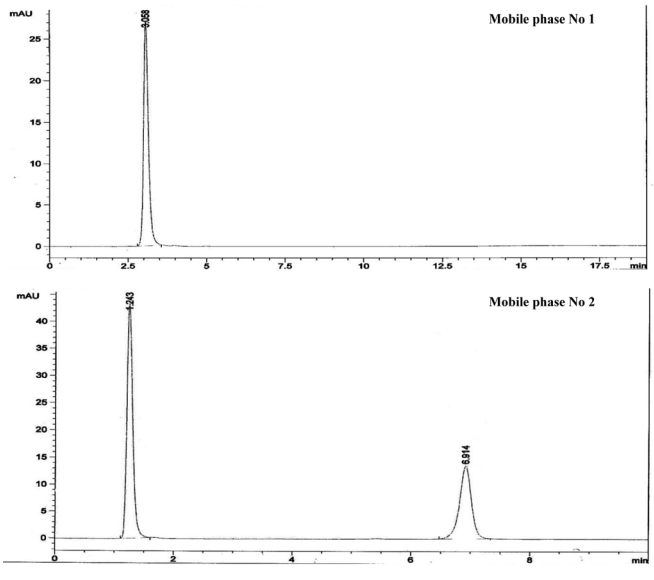

Fig. 4. Chromatogram of mixture at 278 nm and 240nm simultaneously M/Ph. N1 and M/Ph No 2

Furthermore, there were found the good shape of principal peaks and good resolution (Rs 36.04), which are shown in table 3.

The mixture of potassium orotate with spironolactone was evaluated in terms of concentration of each APIs from the mixture, according to the equation (1), relative standard deviation (RSD) and coefficient of variance (CV), which are shown in table 4 and table 5.

The chromatographic techniques developed and reported in this study will serve as a basis for selecting the efficient method for separation and quantitative determination of both APIs simultaneously.

Therefore, it was determined that the concentration of spironolactone is 98.1% (RSD 0.21, CV 0.2%), obtained using mobile phase N2, and the concentration of potassium orotate is 91.68% (RSD 0.15, CV 0.16%), obtained using mobile phase N1. By undertaking of both chromatograms (fig.4) it was found that potassium orotate showed the good resolution from spironolactone on mobile phase, in which spironolactone is predominantly determined.

The method was found to be rapid as potassium orotate and spironolactone eluted out at 1.24 and 6.9 minutes respectively, which is important for routine analysis.

Table 3

Evaluation data of peaks from the mixture on mobile phase No 2

	Theoretical plate (N)		Symmetry (S)		Width (min)		Resolution factor (Rs)	
	Orotate K	Spironolac.	Orotate K	Spironolac.	Orotate K	Spironolac.	Orotate K	Spironolac.
	2259	17147	0.65	0.97	0.1046	0.2112	35.92	
	2266	17713	0.65	0.96	0.1044	0.2072	36.27	
	2272	17227	0.66	0.96	0.1044	0.2101	35.93	
Mean. $\bar{X}\bar{X}$	2266	17362	0.65	0.96	0.10	0.21	36.04	
RSD. %	6.16	306.05	0.004	0.005	0.0001	0.002	0.20	
CV.%	0.27	1.76	0.62	0.49	0.11	0.99	0.56	

Table 4

Evaluation data of mixture on mobile phase No 1

	Retention time (t_R)	Area of peak (A)	Concentration of Orotate K, %
	3.06	1121.683	91.80
	3.058	1118.193	91.514
	3.059	1120.589	91.71
Mean. $\bar{X}\bar{X}$	3.06	1120.16	91.68
RSD. %	0.00	1.79	0.15
CV.%	0.03	0.16	0.16

Table 5

Evaluation data of mixture on mobile phase No 2

	Retention time (t_R)		Area of peak (A)		Concentration %	
	Orotate K	Spironolac.	Orotate K	Spironolac.	Orotate K	Spironolac.
	1.243	6.914	295.94	188.808	75.29	98.15
	1.242	6.894	295.413	189.018	75.15	98.26
	1.244	6.894	296.097	188.269	75.33	97.87
Mean. $\bar{X}\bar{X}$	1.24	6.90	295.82	188.70	75.26	98.10
RSD. %	0.001	0.01	0.36	0.39	0.09	0.20
CV.%	0.07	0.17	0.12	0.20	0.12	0.20

Conclusions

In the present study the compatibility between potassium orotate and spironolactone in the mixture was carefully investigated by applying HPLC method.

Results demonstrated that there was no interaction between potassium orotate and spironolactone in the mixture, due to the absence of any additional peak in the chromatograms, which were investigated by HPLC method. Measurements were performed on RP-18 reversed column (250*4 mm, 5 μm), using mobile phase of acetonitrile: phosphate buffer solution with the ratio 50:50 (pH=4), at flow rate 1.5 mL/min, injection volume 20 μL, temperature 40°C. By analyzing obtained HPLC chromatograms of the potassium orotate in the combination with spironolactone there were shown just 2 principal peaks at 1.24 min with the 278 nm detection wavelength, which is specific for potassium orotate and at 6.9 min with the 240 nm detection wavelength, which is specific for spironolactone. Moreover, potassium orotate showed a good resolution from spironolactone: Rs 36.04. Potassium orotate did not react with spironolactone, therefore the interaction wasn't observed by HPLC. There was found the concentration of spironolactone (98.1% ±0.21), applying mobile phase N2, and the concentration of potassium orotate (91.68% ± 0.15), applying mobile phase N1.

Thus, due to this method a conclusion was made that potassium orotate is compatible with spironolactone. Furthermore, these results were confirmed by FT-IR spectroscopy.

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