

## Fast, simple HPLC method for determination of Spironolactone related compounds

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### Introduction

Spironolactone is chemically  $7\alpha$ -Acetylthio- $17\alpha$ -hydroxy-3-oxopregn-4-ene-21-carboxylic acid  $\gamma$ -lactone. Spironolactone is a steroid and is renal competitive aldosterone antagonist which belongs to the class called potassium-sparing diuretic. Spironolactone acts initially via competitive binding of receptors at the aldosterone-dependent sodium-potassium exchange site. This antagonism effect increases the excretion of water and sodium, while decreasing the excretion of potassium (K<sup>+</sup>-sparing diuretic). Due to this mechanism Spironolactone acts as a diuretic and also as an antihypertensive drug and is indicated for the treatment of congestive heart failure, oedema and ascites in cirrhosis and primary hyperaldosteronism. It is also used for treating hair loss and acne in women, adult acne vulgaris and can be used as a topical medication for treatment of male baldness (Hegazy et al., 2011).

We develop and optimize an analytical method for the determination of related substances of Spironolactone that will be time efficient, robust, and with proven performance and the possibility of efficient separation of Spironolactone from the related substances listed as requirements in the monograph for Spironolactone in the European Pharmacopoeia (European Pharmacopoeia, 2023).

### Materials and methods

The reagents that have been used are: tetrahydrofuran purchased from Carlo Erba, methanol and acetonitrile procured from Merck, Darmstadt, Germany, and the

demineralized water was ‘in house’ prepared with conductivity of 0.05  $\mu$ S/cm.

Valid batches of reference standards: Spironolactone for system suitability CRS, Spironolactone CRS and canrenone CRS were supplied by European Directorate for the quality of Medicines (EDQM). The Spironolactone active substance was obtained from Replek Farm Ltd.

All sample and standard solutions were prepared in accordance to the method for testing Related substances from European Pharmacopoeia monograph for Spironolactone active substance (European Pharmacopoeia, 2023).

Three HPLC systems were used: UPLC Shimadzu Nexera XR LC-40 system with LPG quaternary pump with degasser, autosampler, controller and PDA detector and column oven, controlled by Lab Solutions software, Dionex Ultimate 3000 UHPLC system controlled by Chromeleon software, version 6.80, composed of quaternary LPG pump, autosampler, column compartment and four channel UV-Vis detector and Agilent 1260-II with Open Lab-ChemStation with quaternary LPG pump, autosampler, column compartment and PDA detector. The analytical balance Mettler Toledo AG285, and IKA orbital shaker KS 260 basic were used.

The separation was accomplished using Zorbax C18 Extend 250 x 4,6 mm 3,5  $\mu$ m and Waters Symmetry C18 150 x 4,6 mm 3,5  $\mu$ m, from the group of fully porous particles columns, and, Poroshel C18 Ec 150 x 4,6 mm and 100 x 4,6 mm, 2,7  $\mu$ m particles, all purchased from Agilent and Shimadzu Nex Leaf SH-SPP 150 x 4,6 mm 2,7  $\mu$ m supplied from Shimadzu.

## Results and discussion

The developed method uses binary mobile phase composition of acetonitrile and water and the analysis was performed on different columns.

The concept of mobile phase showed to be important for targeted successful separations of related substances, and afterward change in column selection from columns with lower separating performances like Zorbax C18 extend 250 x 4,6 mm, 3,5 µm and Symmetry C18 150 x 4,6 mm, 3,5 µm, at changed UV monitoring at 240 nm for better sensitivity, contributed shorter run times of about 14 minutes and about 10 minutes alternatively, within the acceptance criteria for system suitability. Even with these standard HPLC fully porous particle columns, we succeeded to reduce run time compared with official European Pharmacopoeia method of 60-65 min, for about 4-5 fold.

When we applied test on superficially porous, or solid core particles columns, with diameter particle of 2,7 µm, and from two different vendors, Agilent Poroshell C18 100 x 4,6 and 150 x 4,6 mm, and the other one product of Shimadzu, Shimadzu Nex Leaf 150x4,6mm, we achieved confirmatory separations of peaks with good peak shapes and sizes, within 5 minutes, 8 minutes, 5.5 and 6 minutes respectively.

These chromatographic conditions generated the same appearance of the chromatogram regarding the retention times of Spironolactone and its impurities. Using the method prescribed in the Ph. Eur. monograph for Spironolactone, the order of elution of the substances of interest is with the following relative retention time: impurity A 0.952; impurity F 1.246; impurity C 1.532; impurity D 1.628; impurity E 1.721; impurity I 1.962. Using the new developed method the obtained elution order is: impurity A 0.947; impurity F 1.222; impurity C 1.514; impurity D 1.658; impurity E 1.770; impurity I 1.974. The separation between all substances of interest is satisfying and complete, with peak-to-valley ratio above 1.5 between impurity A and the peak of spironolactone

Analysis of Spironolactone active substance was performed using the method from the European Pharmacopoeia monograph for Spironolactone and the new developed method. The obtained results for impurities of Spironolactone were comparable between the both methods, with relative difference of each obtained result of each specified impurity lower than 2%.

## Conclusion

The advantages of the new developed isocratic HPLC method for determination of related substances of Spironolactone are the following :

- Use of binary mobile phase with shorter run time, versus the quarterly mobile phase described in the monographs of Spironolactone in European Pharmacopoeia;

- Using different columns that contribute to reducing the duration of the analysis;

- Lower working backpressure using the new developed method, using the pharmacopoeia prescribed HPLC column and chromatographic conditions.

## References

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