Spironolactone: stability indicating method for the identification of major degradation products and pathways

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Introduction

Spironolactone is a potassium-sparing diuretic, used in various diseases such as high blood pressure or hyperaldosteronism. In this study, we developed a stability indicating method and performed a forced degradation study on this molecule to identify major degradation pathways and degradation products of spironolactone during long term stability study.

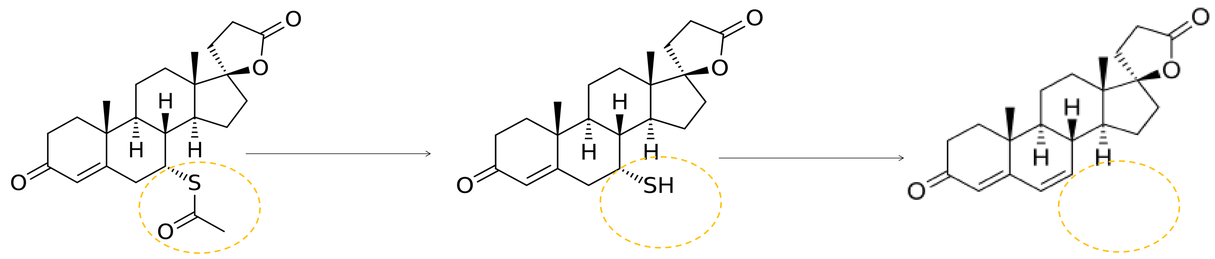

Fig.1 : Major degradation pathway of spironolactone, via alkaline hydrolisis

Material & Method

Forced degradation conditions

Type of stress	conditions
Temperature	60°C – 2 hours
photodegradation	UV lamp – 48 hours
oxidation	H2O2 30 % - 24 hours
Acidic	HCl 0,1 M – 24 hours
Basic	NaOH 0,01 M – 15 minutes
Neutral pH	5 days

Objectifs

- Approximatively 15 % of degradation
- Pure peak of spironolactone
- Pure peak of the majors impurities
- Mass balance conserved

Final chromatographic conditions

- Column: Luna phenyl hexyl (3 µm, 150 × 4.6 mm)
- Mobile phase: 63 water / 30 acetonitrile / 7 methanol (v/v/v)
- Time of run: 55 min
- flow rate of 1.5 mL/min
- Wavelenght: 254 nm and scan rate

Conclusion

The stability indicating method developed allows the elution of spironolactone as a spectrally pure peak, and well resolved from its degradation products. The two main degradation products are well separated (RS>1,5) allowing their evaluation during long term study.

Results & Discussion

Type of stress	conditions	degradation of spironolactone (%)	Sum of impurities (%)	Pure peak of spironolactone / of the impurities
Temperature	60°C – 2 hours	15	10	Yes / yes
photodegradation	UV lamp – 48 hours	7	0,4	Yes / yes
oxidation	H2O2 30 % - 24 hours	12	2	Yes / yes
Acidic	HCl 0,1 M – 48 hours	15	12	Yes / yes
Basic	NaOH 0,01 M – 15 minutes	11	10	Yes / yes
Neutral pH	5 days	12	11	Yes / yes

Table 1. Results of the forced degradation study

Forced degradation conditions were optimized to avoid more than 15 % of spironolactone degradation, in order to limit secondary degradations.

Mass balance is approximatively conserved in the case of stress induced by temperature and acidic neutral or basic conditions. Canrenone, one of the major degradation impurity have a maximum of absorption at 280 nm which can explain the small differences in mass balances observed

In case of stress induced by oxidation (H2O2 and photooxidation), the mass balance is incorrect: the degradation products are not observed via the developped methd. But oxidation appears not to be the major degradation patway

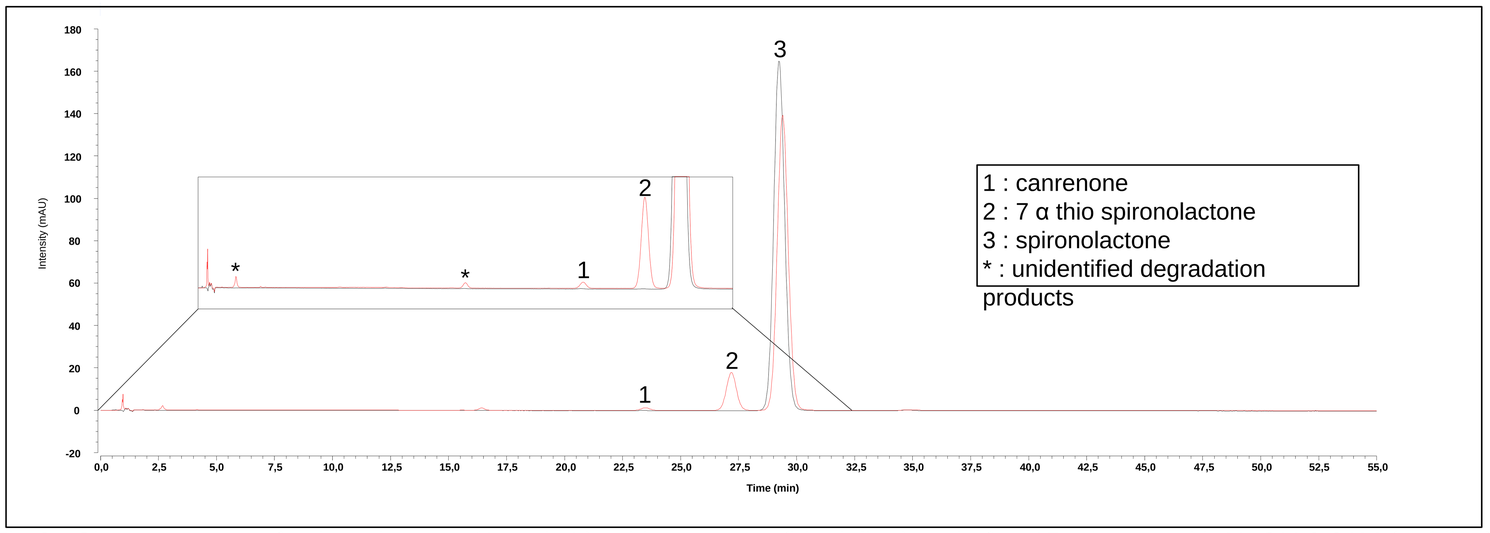

Fig.2 : Chromatogram of spironolactone (1 mg/mL) without stress (black line) and after 15 minutes with NaOH 0,01M (red line)

The LOD and the LOQ (0,3 µg/mL and 0,9 µg/mL respectively) enable the detection of impurity at 0,05 % of spironolactone (ICH recommandations).

The two main degradation products : canrenone and 7 α thio spironolactone are well separated (Rs > 1,5).

For all type of stress, the chromatographic peaks are pure

Some degradation products formed in stressed conditions are observed but not identify yet. If these impurities are observed during stability studies, there identification will be needed.