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## Enantiomeric separation of (-) and (+)-hyoscyamine by High Performance Liquid Chromatography-Tandem Mass Spectrometry

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J. Marín-Sáez<sup>1\*</sup>, R. Romero-González<sup>1</sup>, J. L. Martínez-Vidal<sup>1</sup>, R. López-Ruiz, A. Garrido-Frenich<sup>1</sup>.

<sup>1</sup> University of Almería, Department of Chemistry and Physics, Research Centre for Agricultural and Food Biotechnology (BITAL), Agrifood Campus of International Excellence ceiA3, Carretera de Sacramento s/n, E-04120 Almería, Spain.

\*jms485@inlumine.ual.es, Tel: 670351603

Interest in natural toxins produced by fungi or plants has grown in recent years due to its toxicity and its impact on feeding and animal feed and human food safety. Among these toxins, alkaloids and more specifically tropane alkaloids, are very significant and they are characterized by presenting anticholinergic activity. Tropane alkaloids are synthesized by the plants from *Solanaceae* family and in smaller quantities from plants from other families. This family of compounds involves more than 200 compounds, although the compounds most studied are scopolamine, atropine and (-)-hyoscyamine [1].

In the case of atropine, which is a racemic mixture of (-) and (+)-hyoscyamine, it is well known that (-)-hyoscyamine exhibit stronger anticholinergic effects than the (+)-hyoscyamine. Moreover, racemization occurs during the treatment process of the raw material and cooking procedures due to pH and temperature modifications, since only the (-)-hyoscyamine is naturally formed. For this reason, according to the EFSA interest in 2013 [1] it is necessary to develop methods which allows the separation of these two chiral enantiomers to elucidate the conditions that favor the transformation between both enantiomers, because there are not many studies that evaluate these processes.

Therefore the aim of this study is the optimization of the enantioselective separation of (-) and (+)-hyoscyamine with a Chiralpak-AY3 (150x4.6 mm, 3 µm) analytical column in combination with high performance liquid chromatography (HPLC)-coupled to tandem mass spectrometry (MS/MS) has been used. The developed method allowed the separation of the two target compounds in 10 min, using ethanol 0.1% diethanolamine as mobile phase. In addition, the influence of different parameters, such as pH apparent or temperature, in the signal of the (-) and (+)-hyoscyamine was studied. Finally the method was applied to the determination of the target compounds in buckwheat samples. Performance characteristics, such as trueness (in terms of recovery), precision, linearity, detection limits (LODs) and quantification limits (LOQs) were studied. Suitable performance characteristics were obtained for a reliable determination of both enantiomers in the selected samples.

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### References

[1] EFSA. Scientific opinion on tropane alkaloids in food and feed, EFSA panel on contaminants in the food chain (CONTAM). *The EFSA Journal*, **11**, 3386-3499 (2013).