

Enantiomeric determination of atropine in *Datura Stramonium* and *Brugmansia Arborea* seeds by LC-MS

Solanaceae family plants can contain huge amounts of tropane alkaloids, which have anticholinergic activity. There are more than 200 tropane alkaloids but most studies are mainly focused on atropine and scopolamine determination. Although animals can also be affected by these compounds, they are less susceptible than humans to be poisoned by alkaloids, because the presence of an esterase enzyme, which efficiently biotransforms the atropine. Besides, atropine is a mix of two enantiomers, (+,-) hyoscyamine, and only (-)-hyoscyamine has anticholinergic activity. The European Union has recommended the development of methods that allow for the enantioseparation of atropine. This feature is also very important for quality control in the pharmaceutical industry in order to achieve a better drug which should be enantiomerically pure, improving its efficiency.

Although information related to the racemization process is scarce, it is well-known that the extraction procedure can affect the ratio between both isomers, and therefore, the extraction conditions should be deeply studied in order to minimize this transformation process. Therefore, to achieve reliable results a chiral separation of these compounds must be created.

In this study a new chiral method for the determination of (+,-) hyoscyamine has been developed, applying a rapid QuEChERS method that prevents the racemization process in the extraction step and a Chiralpak AY3 column for the chromatographic resolution. The mobile phase used was ethanol containing 0.1% of diethanolamine (DEA) at flow rate 0.4 mL/min. The analysis was carried out by a mass spectrometry triple quadrupole analyser that provides low limits. Once the method has been developed, it was applied to the analysis of real samples of *Solanaceae* seeds: *Datura Stramonium* and *Brugmansia Arborea* seeds, as well as contaminated buckwheat samples (mixing 1 g of *Solanaceae* seed with 1 kg of buckwheat), obtaining only the active enantiomer (Tab. 1).

Sample	(-)-hyoscyamine	(+)-hyoscyamine
Datura seeds	1454.30 (7) ^a	16.21 (11)
Brugmansia seeds	326.91 (9)	17.88 (9)
Buckwheat contaminated with <i>Stramonium</i>	0.17 (15)	0.01 (12)
Buckwheat contaminated with <i>Brugmansia</i>	0.10 (6)	0.01 (3)

Tab. 1. Concentrations (mg/kg) of (+,-) hyoscyamine in the tested samples

^a Relative standard deviation is given in brackets (n = 4)

Then the racemization process was studied under pH and temperature conditions, noting that the combination of high temperatures and basic pHs provides the conversion to the inactive enantiomer, whereas when the only contribution of pH or temperature was studied, the interconversion rate reached until 80% (Fig. 1).

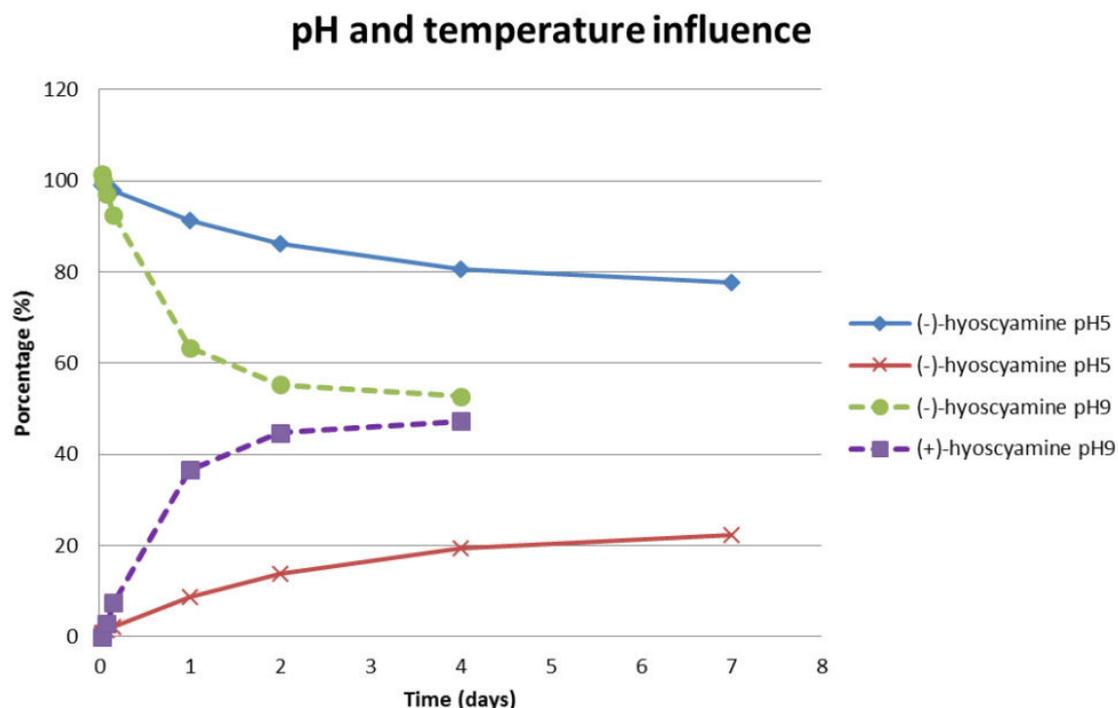


Fig. 1. pH and time influence in the enantiomerization of (-)-hyoscyamine in *Stramonium* seeds at 80 °C.

In conclusion, due to the shortage of enantiomeric method for the determination of atropine, a new chiral method has been developed. This method has been applied to real *Solanaceae*s seeds and contaminated samples obtaining only the active enantiomers. Then, different pH and temperature conditions were probed only obtaining a complete racemization with basic pH and high temperatures, concluding that in a real contamination with *Solanaceae*s seeds, the enantiomer with pharmacology activity prevail against the inactive enantiomer.

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