CHROMATOGRAPHIC AND DEGRADATION STUDIES OF *FRANGULA* EMODIN

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Introduction

Frangula emodin, (1,3,8-trihydroxy-6-methyl-anthraquinone), is one of the anthraquinone derivatives found abundantly in the roots and bark of a number of plant families that have been used traditionally to treat constipation and haemorrhoids for hundreds of years. It is also known to have antimicrobial, antiviral, antifungal, anti-inflammatory, antioxidant, and immunosuppressive activities. Extensive research is being dedicated to this compound as an anti-cancer drug and it is also showing great potential as a treatment for type 2 diabetes. (Leng, 2010)

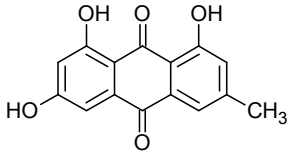

Figure 1: Structure of emodin

Methodology

A HPLC Stability Indicating method for *Frangula* emodin was developed. Selectivity was demonstrated by performing forced degradation studies to produce samples that contained the significant and relevant degradation products. Peak purity testing was performed on the resultant chromatograms in the range of 200 to 600 nm with a resolution of 1.2 nm to determine whether the prominent peaks were spectrally pure or not. Identification of the eluted degradation peaks was attempted. The method was validated for its linearity, accuracy, precision, selectivity, range, LOD and LOQ as per ICH guidelines at both an assay and degradation product level. A system suitability test was also carried out to evaluate the reproducibility of the analytical system using five replicate injections of a standard solution.

Results & Discussion

Instrumentation	HPLC Water Alliance 2695 Separations Module
Column	Waters Symmetry C18 250 x 4.6mm, 5µm
Mobile Phase	Mobile Phase A: 0.01% TFA 0.1% Formic Acid Mobile Phase B: 100% Methanol HPLC grade
Gradient Profile	

	Time (mins)	%A	%B
1	0.01	35	65
2	10.00	35	65
3	20.00	28	72
4	31.00	15	85
5	40.00	10	90
6	41.00	35	65

Flow rate	1.00 mL/min
Run Time	41 minutes
Column Temperature	35 °C
Sample Temperature	4 °C
Injection volume	10 µL
UV detection wavelength	287 nm, and PDA Analysis (210-600 nm)
UV Resolution	1.2 nm
Diluent	100% Methanol
Solution Filters	0.45µm GHP filters
Test concentration	0.5 mg/mL

Table 1: Final chromatographic parameters for the validation of the SIM of emodin

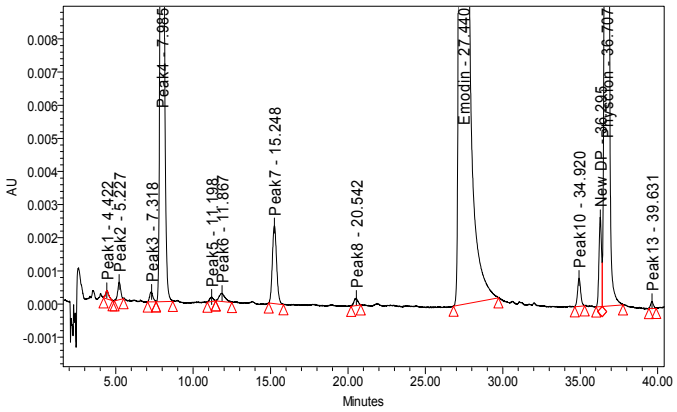

Figure 2: Chromatogram of emodin stress solution under basic conditions

The Stability Indicating Method of emodin was developed and validated successfully. The chromatographic parameters are shown in Table 1. Emodin eluted at around 27.4 minutes. Physcion was the only identified related substance and it eluted at around 36.7 minutes. Forced degradation studies highlighted the susceptibility of emodin to basic conditions. The loss in percentage assay of emodin was accompanied by a change in colour from orange-yellow to red and also by the elution of a new peak at 36.2 minutes. The observed changes are believed to be a result of tautomerization of emodin (Fain *et al.,* (2005); Mohanlal *et al*., (2011)).

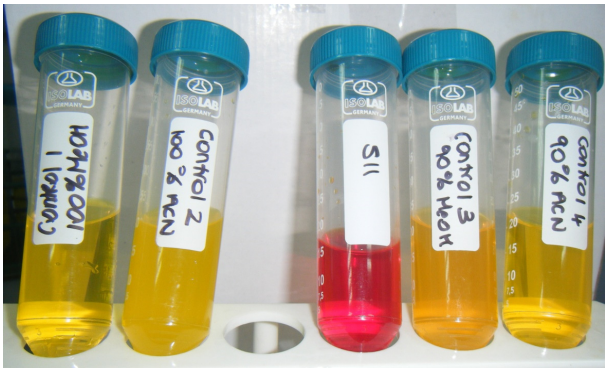

Figure 3: Photograph of emodin base hydrolysed stressed solution (S11) and corresponding controls

References

1. Fain, V. Y., Zaitsev, B., & Ryabov, M. (2005). Tautomerism of the natural anthraquinones physcion and emodin and their analogs. Chemistry of Natural Compounds , 501-507.
2. Mohanlal, V., Steenkamp, P., & Odhav, B. (2011). Isolation and characterization of anthraquinone derivatives from Ceratotheca triloba (Bernh.) Hook.f. Journal of Medicinal Plants Research , 3132-3141.
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