Quantitative determination of aescin in combined *Aesculus*/*Vitis* capsules

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"Aescin", the saponin mixture obtained from the seeds of *Aesculus hippocastanum* L. (horse chestnut), is widely used in the treatment of peripheral vascular disorders. This mixture of saponins has been reported to show anti-inflammatory activity [1,2] whereas structural requirements of aescins for this activity were obtained by Matsuda *et al.* [3] by investigating the effects of the pure saponins on acute inflammation in experimental animal models.
Capsules containing *Vitis vinifera* L. extract in combination with *Aesculus* extract will be marketed by the firm Biover (Brugge, Belgium) to treat peripheral insufficiency and hemorrhoids. A method for quality control on the saponin content in both the extract and the capsules needed to be developed.
For aescin analysis according to the German Pharmacopoeia (DAB) a colorimetric method is used. The amount of aescin is determined by measuring the absorbance at 540 nm after converting all the saponins into the same colored molecule by reacting with ferrichloride/acetic acid/sulfuric acid reagent. Due to interference of constituents (polyphenols) of the *Vitis* extract, this non-specific assay could not be applied to the capsules. Several HPLC [4-6] and TLC-densitometric [7–9] methods have been suggested for quality control of aescin and single *Aesculus* preparations. Due to the poor UV absorbance of triterpene glycosides, it was preferred to use HPTLC after spraying with anisaldehyde reagent in favour of HPLC-UV. In order to eliminate the interference caused by the *Vitis* extract a sample preparation step using C-18 SPE cartridges was included. The final method was validated according to the ICH guidelines and shown to be linear, repeatable and accurate.

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References:
[1] Rothkopf M. and Vogel G. (1976) *Arzneimittelforschung* **26**, 225-235.
[2] Vogel G. *et al.* (1970), *Arzneimittelforschung* **20**, 699-700.
[3] Matsuda H. *et al.* (1997) *Bioorganic & Medicinal Chemistry Letters* **7** (13), 1611-1616.
[4] Wagner H. *et al.* (1985) *Deutsche Apotheker Zeitung* **125** (30), 1513-1518.
[5] Pietta P. *et al.* (1989) *Journal of Chromatography* **478**, 259-263.
[6] Profumo P. *et al.* (1991) *Planta Medica* **57**, 50-52.
[7] Hammerstein F. and Kaiser F. (1972) *Planta medica* **21**, 5-15.
[8] Uberti E. *et al.* (1990) *Fitoterapia* **61** (1), 57-60.
[9] Vanhaelen, M. and Vanhaelen-Fastre, R. (1983)