B005 Polymer based affinity modules for modern phytochemical analysis

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Most extracts derived from nature are highly complex mixtures of various compounds. But quality, efficacy and also toxicity of those extracts used for pharmaceutical purposes are often related to a small number of substances. Using newly developed affinity modules, analysis of compounds of interest can be performed out of a crude extract. These modules are based on polymeric materials and carry an immobilized bio-component, which is responsible for the specific recognition of a target molecule. In recent years, several immobilisation techniques for enzymes, antibodies, glycosides and other molecules were published (1), but these methods refer to expensive base-materials (*e.g.*, polymeric carbohy-drates, silica-materials). The aim of investigations described here was the development of inexpensive and multifunc-tional affinity modules for a rapid analysis of single compounds.

Poyethylene and polypropylene were used as porous polymeric materials. After activation of the surface, a spacer was attached followed by covalent coupling of a specific affinity-group (2,3). This affinity-group is able to interact with defined molecules like lectins, avidin or metal-chelating proteins (Figure 1). The latter molecules are coupled to the biologic recognition elements as antibodies, antigens or DNA, which have already been developed. The modules can be used for phytochemical analysis, detection of microorganisms in pharmaceutical preparations, and DNA-analysis. Systems for detection of pyrrolizidine alkaloids and detection of microbial contaminants (*e.g. Salmonella*) are under current testing.

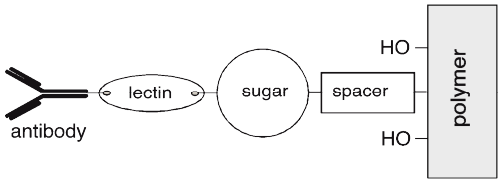

Figure 1. Coupling of an antibody via lectin-sugar interactions.

References: 1. Srere, P.A. and Uyeda, K. (1976) Meth. Enzymol. 44, 11-19. **2.** Keusgen, M. et al. (2001) Biotechnol. Bioeng. 72, 530-540. **3.** Milka, P. et al. (2000) Biotechnol. Bioeng. 69 (2000) 344-348.

B006 Comparison of photometric and HPLC-ELSD analytical methods for *Tribulus terrestris*

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The aerial parts of *Tribulus terrestris* have been used to manufacture the Tribestan product which has been used to treat male and female sexual disfunctions, this action is attributed to the steroidal saponins protodioscin and protogracillin. A photometric method of analysis (1) has been used by this manufacturer and adopted by other manufacturers in Bulgaria. The saponins have very poor UV absorption and are unable to be determined by HPLC-DAD, a recent report has outlined the determination of steroidal saponins by RP-HPLC with ELSD detection (2). A comparison of the photometric method and the HPLC-ELSD shows that the photometric method is unable to accurately measure the level of protodioscin and related compounds in even an ideal Tribulus preparation. The poor specificity of the photometric method leads to increasingly more inaccurate results once the sample constituents vary. A wide variation in the saponin distribution of drug samples of different geographical and plant part has been found and has shown that the photometric method responds to a range of compounds, not just protodioscin, samples with no protodioscin or related saponins still respond to this method. In samples with no protodioscin the absorption spectrum is markedly different to that obtained with protodioscin.

With the ready availability of reference standard materials of high purity and confidence, adoption of the HPLC-ELSD as the preferred method of analysis of *Tribulus terrestris* products is strongly recommended.

References: 1. Gjulemetowa, R. et al, (1982) Pharmazie, 37: 296. **2.** Ganzera, M. et al, (2001) J. Pharm. Sci., 90: 1752-1758.