**Improving the simulated moving bed separation of oleanolic and ursolic acids with a C30 stationary phase**

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**Highlights**

* C30 column and methanol/water were best phases to separate oleanolic and ursolic acids.
* Equil. & mass transfer constants of pure compounds were validated for binary mixture.
* An SMB using C30 columns is capable of separating the two acids with purities of 99.9 %.
* This is a significant improvement over previous results obtained with C18 columns.

**1. Introduction**

*Eucalyptus* *globulus* is a predominant species in the Portuguese forest [1] and a vital resource for the pulp and paper industry. The bark residues are usually burned for energy production without any further valorization. Recently, the bark has been identified as a source of triterpenic acids (TTAs) such as oleanolic and ursolic acids [2]. These compounds are known to possess a wide spectrum of bioactivities, including anti-oxidative, antitumoral, anti-inflammatory, anti-hyperlipidemic, and anti-microbial effects [3]. Thus, under the scope of the biorefinery concept, *E. globulus* bark is a potential candidate to extract high-value compounds such as TTAs. However, their separation after extraction is challenging as oleanolic and ursolic acids are two structurally related isomers and occur simultaneously in the same natural matrix. Simulated moving bed (SMB) chromatography is a continuous adsorption technique, which appears as an efficient alternative to batch elution chromatography. The SMB continuous countercurrent mode of operation maximizes the mass transfer driving force, providing improved productivity and reduced solvent consumption [4]. Thus, an SMB process may be a potential candidate for TTAs separation.

**2. Methods**

A series of impulse experiments were conducted to select appropriate mobile phases for the separation of oleanolic and ursolic acids. Experimental breakthrough curves were measured in a custom laboratorial installation. Breakthrough of pure components were conducted to determine equilibrium and mass transport parameters, by fitting a chromatographic model to the experimental data. Parameters were validated through the successful prediction of breakthrough assays of binary mixtures.

**3. Results and discussion**

From a series of several impulse tests methanol/water 95/5 (%, v/v) emerged as the most favorable mobile phase to conduct their continuous separation by SMB providing a value of selectivity of 1.08. The C30 column demonstrated a remarkable separation capacity for the triterpenic acids, enabling simultaneously higher selectivities and faster analysis times, when compared with previous results using a C18 packing material and the same mobile phase [5]. Equilibrium and mass transport parameters obtained from breakthrough experiments of pure acids (and validated for a binary mixture) were used to design the SMB separation of a representative mixture of oleanolic and ursolic acids from a natural extract of *E. globulus*. A classical SMB scheme was simulated and optimized using a Design of Experiments – Response Surface Methodology. Purity requirements were defined while the productivity was maximized. Rigorous phenomenological simulation results, of which the concentration profile in the SMB at cyclic steady state is presented in Figure 1, demonstrated that the SMB is able to attain purities levels of 99.9 %, for both extract and raffinate outlets, and productivities of 1.705 kg/(m3adsorbent day) with 2-2-2-2 configuration. This is a significant achievement as previous results with a C18 stationary phase showed that purities of 99.4 % and 99.1 % for ursolic and oleanolic acids, respectively, were achievable at the expense of using three columns per section (3-3-3-3), and consequently at the expense of extremely low productivities [5]. The work presented here with the C30 column represents important improvements towards the successful chromatographic separation of these triterpenic acids.



**Figure 1.** Simulation results of SMB operation at cyclic steady state for the isolation of oleanolic (blue line) from ursolic acid (orange line). E – eluent; F – feed; X – extract; R – raffinate.

**4. Conclusions**

The separation of oleanolic and ursolic acids by SMB was enhanced by applying C30 columns and a methanol/water 95/5 (%, v/v) mobile phase. The SMB unit with two columns per section, which was optimized combining the design of experiments and response surface methodologies with phenomenological computer simulations, attained purities of 99.9 %. This represents a significant improvement in terms of purity and productivity when compared with previous results obtained with C18 columns, which required a 3-3-3-3 configuration to attain purities of 99.4 %.

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