

Industrial continuous enrichment of *Aloe vera* extract by True Moving Bed CPC/CCC [1]

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Aloe Vera is a stem less or very short-stemmed succulent plant growing up to 60-100cm in height spreading by offsets. It is common practice for cosmetic companies to add soap or other derivatives from *Aloe Vera* in cosmetics products.

Figure 1 present the HPLC chromatogram of the crude *Aloe vera* extract. The aim of this study is to enrich the extract in target molecule (R_t : 3.02 mn) and to remove all traces of two major impurities (R_t : 13,7 and 14,3 mn) continuously using an Armen TMB CPC¹[1].

¹ True Moving Bed Centrifugal Partition Chromatography

Keywords: TMB, *Aloe vera*, CCC, continuous enrichment

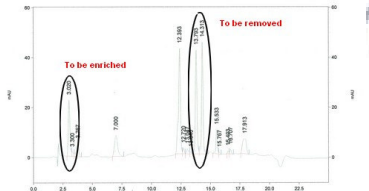
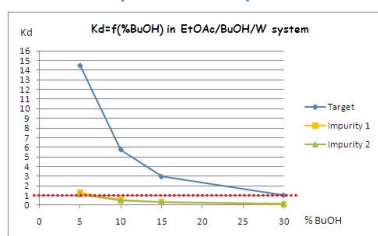


Figure 1: HPLC chromatogram of crude *Aloe vera* extract

TMB principle and solvent system determination

Principle of True moving Bed process [Fig. 2]:

- The Armen TMB [Fig. 7] is equipped with two columns (rotor 1 and 2), two elution pumps: two injection pumps, two fraction collectors. All peripherals are using alternatively in ascending and descending mode
- Both rotors are filled with 50% of each phase.
- The sample, dissolved into both phases is continuously injected between the two rotors. In descending mode as the mobile phase is the lower phase and in ascending mode as the mobile phase is the upper phase.
- The collections of the molecules A and B is done alternatively during each cycle.
- Cycle numbers are determined according to the volume of sample to purify, injection flow-rate and elution flow-rate
- Molecule B which get more affinity for upper phase ($K_d[\text{upper}]/[\text{lower}] > 1$) will be eluted in ascending outlet and molecule A which get more affinity for lower phase will be eluted in descending direction ($K_d[\text{upper}]/[\text{lower}] < 1$)



Graph 1: K_d of impurities and target compound according to butanol content in EtOAc/BuOH/W system. K_d are measure by the shake flask method in HPLC

Solvent system determination.

According to the polarity of the extract, the solvent system ethyl acetate/butanol/water was tested with different proportion of butanol. K_d are measuring with the shake flask method for each solvent system. Results obtained are resuming in graph 1. In this case the aim is to continuously enriched an extract in target 1 and eliminate all traces of impurities 1 and 2. A solvent system where target compounds get a $K_d < 1$ and both impurities 1 and 2 get $K_d > 1$ would allows to reach this objectives according to the principle describe above. According to the K_d measurement solvent system EtOAc/BuOH/W 35/15/50 was selected for the TMB run

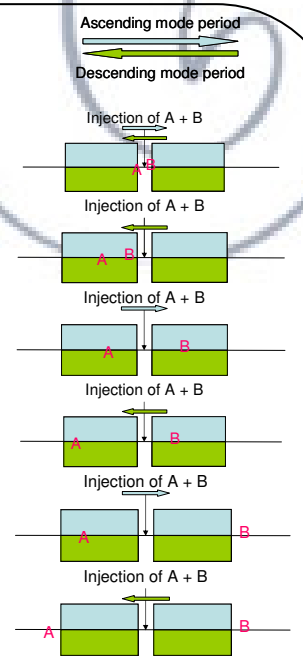


Figure 2: Principle of TMB

TMB run and results

Figure 3: TMB CPC principle. Injection of crude between both CPC column continuously and recovery of enriched extract without any traces of impurities in the lower phase

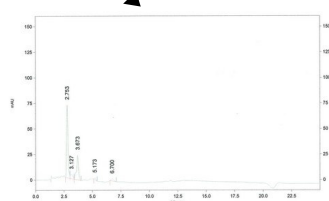
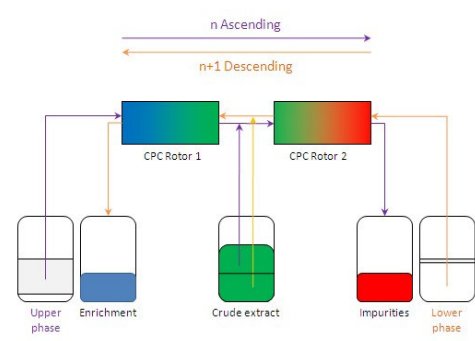


Figure 6: HPLC chromatogram of enriched fraction

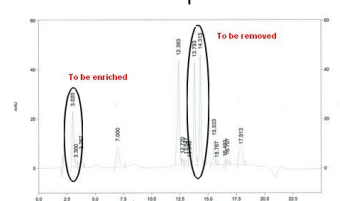


Figure 4: HPLC chromatogram of crude *Aloe vera*

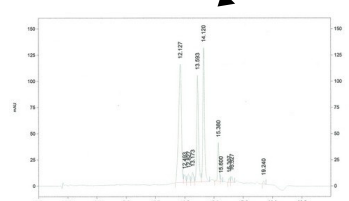


Figure 5: HPLC chromatogram of impurities

TMB run is a continuous injection of the crude sample between the two CPC columns. More polar compounds of the extract (which has more affinity for lower aqueous phase) are recovering from one side of the system and the less polar compounds of the extract (which has more affinity for the upper organic phase) on the other side [Fig. 3]. Figure 4 shows the HPLC analysis of the crude *Aloe vera* extract to be enriched. Figure 5 is the HPLC analysis of the fraction obtained in ascending mode in the upper phase which contains impurities without any traces of target compound. Figure 6 is the HPLC analysis of the second fraction obtained in descending mode in the lower phase which contains the target molecule without any traces of impurity.

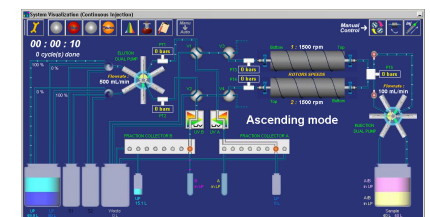


Figure 7: Armen TMB CPC 1L and Armen Glider Software

Conclusion

The difference shown here with a simple biphasic liquid extraction system in funnels, that CPC TMB gives enough separating power in terms of plate efficiency to get two different purified extracts containing different molecules. The size of the CPC TMB columns defines the scale of productivity, for example a 12.5 litre system is capable of delivering kg scale levels of compound and larger systems can further enhance these levels.

[1] TMB patented process IFP, François Couillard