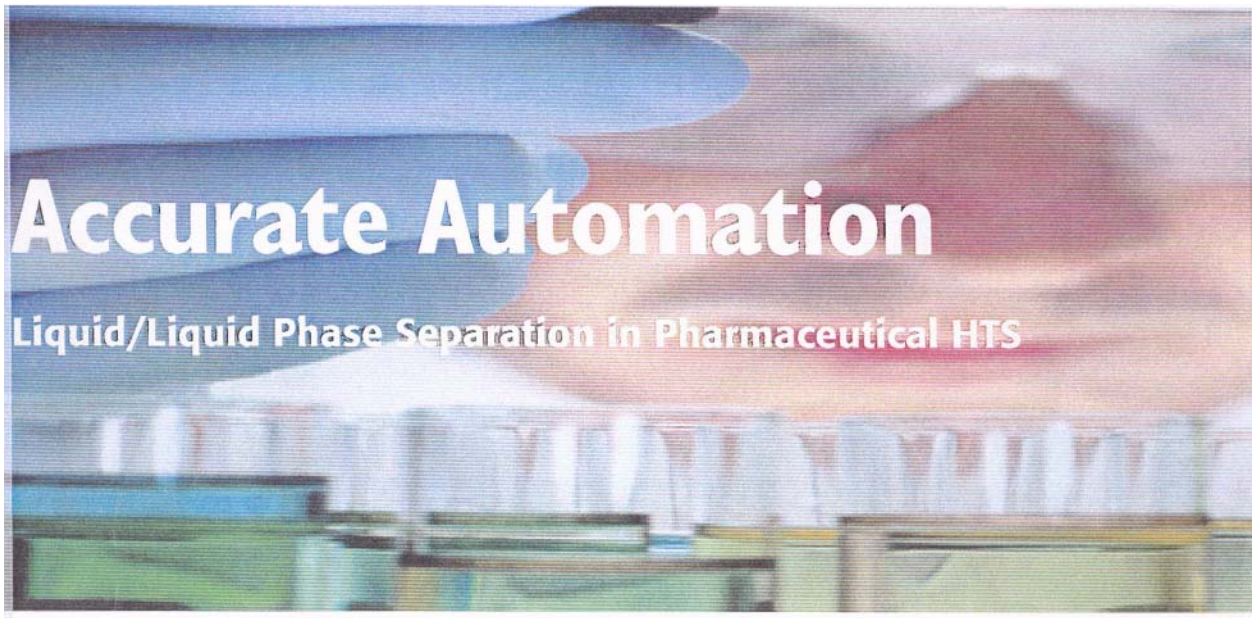



**The distribution of pharmacologically active compounds between organic and aqueous media is a very important criterion in the process of drug discovery. Often, after the high throughput screening has revealed a number of interesting compounds, an organic/aqueous phase distribution assay contributes to the decision whether to do any further testing with the compound or not. Within this assay, an accurate phase separation is of crucial importance, to make sure that in the subsequent quantitative analysis only one phase is present. Here, we present a new and fully automated approach -suitable for high throughput screening — to selectively separate an organic and an aqueous phase using a combination of pressure (pLLD) and capacitive (cLLD) liquid level detection which is independent of the relative volumes of the two phases.**

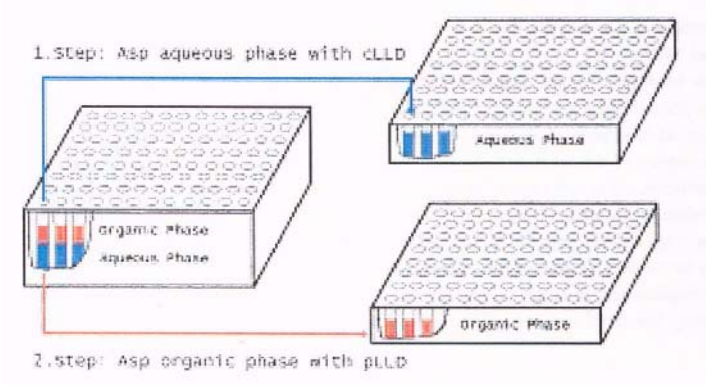



**Figure I: Accurate automated Liquid/liquid phase separation using pressure (pLLD) and capacitive (cLLD) liquid level detection. The figure shows the scheme used in our experiments**

## Equipment Used

The experiment is performed with a HAMILTON Microlab® STAR pipetting work station.The Microlab® STAR is the first multi-channel pipetting device which works on a simple air displacement pipetting principle, like a hand-held pipette, without any system liquid. This opens up the possibility of measuring pressure and temperature within each of the 8 independent and freely spreadable pipetting channels of the robot.The pressure sensor can then be used for pressure-based LLD. To obtain reliable pressure measurements, a tight coupling of the pipetting tips to the pipetting head is of crucial importance. The Microlab® STAR is equipped with CO-RE Technology (for Compression-induced O-Ring Expansion) pipette tips.This approach allows the tip/head connection to be sealed very accurately by means of a compressed O-ring, and thus enables real-time pressure measurements. The liquids used in our experiment are WD40, a lubricating oil as organic phase, and deionized water with 0.1% KG added.

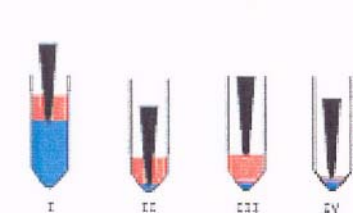
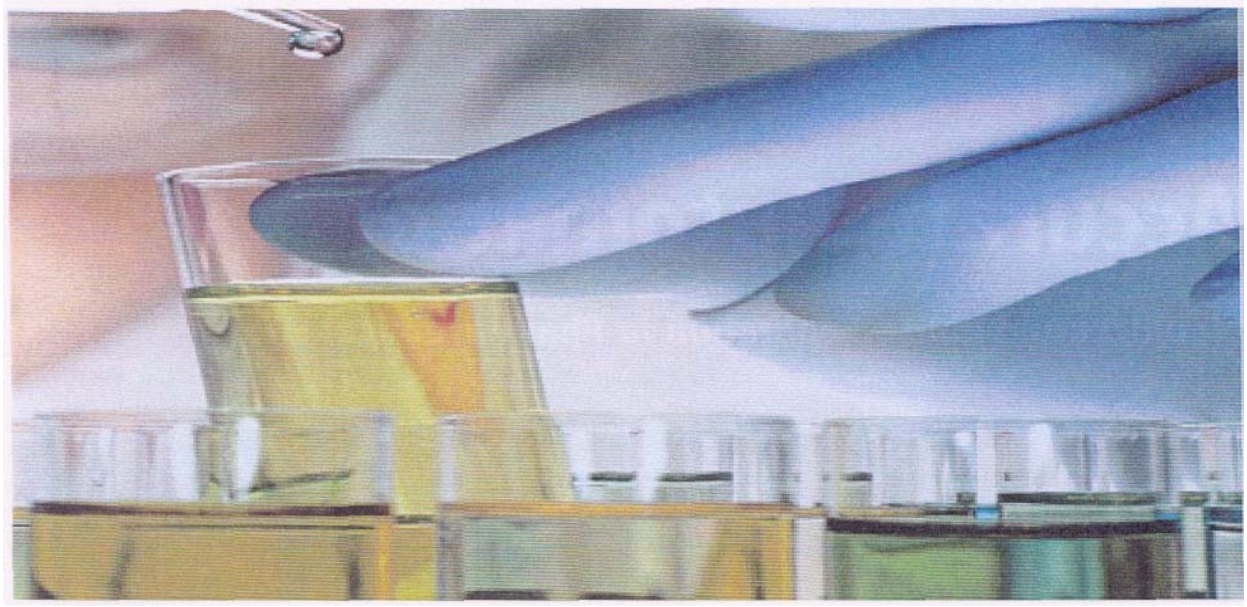



**Figure 2: The aqueous phase is painted in blue, the organic phase is painted in red. In step I, the phase boundary is detected with capacitive LLD. Using a small (lmm) submerge depth, the pure aqueous layer is selectively aspirated in step II following the falling liquid level. In step III, the liquid level of the organic layer is detected by pressure LLD. Again, a small submerge depth (lmm) is used in step IV to aspirate only the organic phase following the falling liquid level**

## Experiment: Description and Results

In our experiment, a 96-deep-well micro-plate is prepared with the two phases (see figure I): the organic layer (OP) on top and the aqueous layer (AP) on the bottom. The two phases are separated into two normal 96-well microplates for analysis of the distribution pattern. In the first step (see figure 2), we use capacitive LLD to accurately detect the phase boundary. This is possible, because on passing through the organic layer, no dramatic change in the capacitance signal is measured. Only when the tip reaches the aqueous layer is a signal change detected, indicating the position of the water surface. Then, a defined volume of the aqueous phase smaller than the total volume $V_{AP}$ of the aqueous phase (V^W,^) is aspirated and dispensed to the corresponding microplate. In the second step, pressure LLD is used to detect the surface of the non-conductive and non-polar organic phase.As for the aqueous layer, a defined volume of the organic layer ($V_{asp}$^VOP) is aspirated and transferred to the second microplate. During both



aspirations we follow the falling liquid level within the well. This procedure now enables a "clean" separation of the two phases, because the aspiration takes place in the bulk of the phases and in the region of the surface boundary. Furthermore, no assumptions about the individual phase volumes have to be made. The experiment results in two accurately separated phases, each stored in a microplate and a small remainder containing the dead volume of the process (about 50 ul per phase) in the original deepwell microplate.

## Conclusion

Our results indicate that an organic and an aqueous phase can be separated very accurately in a high throughput screening application. Using this approach, the distribution assay can be integrated into the first part of the process of drug discovery, where typically large numbers of compounds are screened. In turn, this helps to reduce screening costs, because the result - whether a compound fulfils the distribution criterion or not — can be obtained earlier in the process.

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