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Moving Standardization of HPTLC to the Next Level

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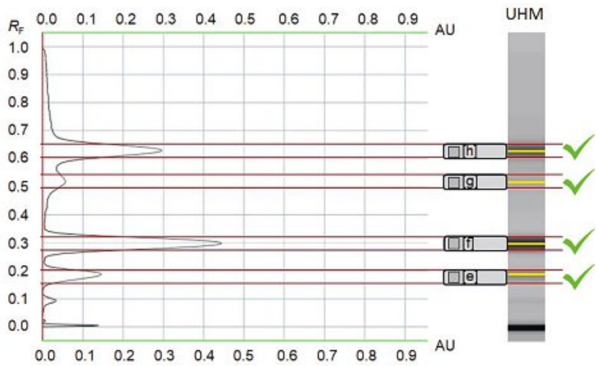
High Performance Thin Layer Chromatography (HPTLC) is a well-known chromatographic separation technique applied in a large number of methods, particularly for generating chemical fingerprints of herbal drugs. Standardized HPTLC methods, focused on reproducibility and qualification of the chromatogram, are the ticket to reliable analytical results in daily routine.

Traditionally, qualification is performed on each plate using an appropriate system suitability test (SST). For that, nearly all methods rely on the chromatographic behavior of specific chemical reference substances, typically related to the target analytes. The search for individual substances is often cumbersome and expensive. Therefore, a generally applicable, cost- and time-efficient alternative for use in the SST was sought.

This led to the development of a novel approach using the Universal HPTLC Mix (UHM), a pre-defined mixture of eight substances. The selection of the substances started with considerations of minimum environmental and health hazards. Then, detectability, stability, availability and price were taken into account. The final mixture includes compounds from a broad polarity range featuring different functional groups.

A large number of chromatographic systems separate the UHM into a defined and unique pattern of zones with specific response characteristics to changes of the activity of the plate, composition of the mobile phase, and degree of saturation of the developing chamber. The use of these patterns in the SST allows checking whether a method was performed appropriately. Based on assigned R_F values and their respective windows obtained during method validation, such SST can be performed automatically. Furthermore, the SST can be used as reference point for adjustments of R_F shifts caused by differences in plate batches and for the normalization of signal intensities between plates. The UHM is now commercially available.

The future of standardized HPTLC.



System Suitability Test (SST) view using the UHM consisting of [e]: phthalimide; [f]: 9-hydroxyfluorene; [g]: thioxanthen-9-one; [h]: 2-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol. Note: the other four substances of the mixture, namely guanosine, sulisobenzone, thymidine, and paracetamol stay at the application position. HPTLC Silica Gel F_{254} glass plate developed in the HPTLC PRO Module DEVELOPMENT, using toluene as developing solvent, and scanned at 254 nm.

The Universal HPTLC mix opens the door to the next level of standardization of HPTLC methods. Software-based system suitability tests and normalization of data are getting within reach, meaning that in the future, results will be highly comparable in-between different laboratories and time periods. This is the first step towards a data-driven HPTLC with focus on artificial intelligence.

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Reference

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