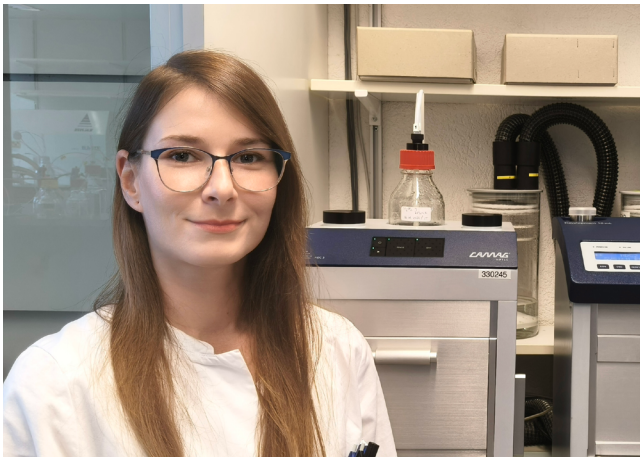
Unified HPTLC screening of photoinitiators and benzophenone derivatives

Sonja Drobnjak



Sonja is a Lab Scientist at CAMAG with over six years of laboratory experience in analytical chemistry and biotechnology. She specializes in HPTLC method development and optimization, and scientific project support. Her work is driven by a strong focus on chromatographic analysis, data quality, experimental design, and solving complex analytical challenges.

Introduction

The increasing use of UV photoinitiators and UV filters in printing inks, coatings, plastics, and cosmetic products has created a growing demand for efficient analytical screening methods. Several compounds, including TPO (diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide) and certain benzophenone derivatives, are receiving increasing regulatory attention due to their potential migration and toxicological concerns, particularly in food contact materials [1].

The analyte panel investigated in this study was selected to broaden the scope of a previously established HPTLC method, by including additional photoinitiators and structurally diverse benzophenone derivatives with different chromatographic behavior. This extended panel therefore provides a broader model for evaluating the suitability of a unified HPTLC screening approach across compounds with markedly different polarity and migration characteristics.

An HPTLC method for the detection of TPO, TPO-L (ethyl (2,4,6-trimethylbenzoyl) phenylphosphinate), and BAPO (phenylbis(2,4,6-trimethylbenzoyl)phosphine oxide) was previously developed and showed good chromatographic performance for these phosphine oxide photoinitiators [2]. However, extending the method to additional compounds, including Irgacure derivatives and several benzophenone UV filters, revealed limitations in selectivity, especially for structurally related benzophenone derivatives.

The development of a single HPTLC method capable of screening twelve reference substances offers significant advantages for routine laboratories. A unified approach simplifies analytical workflows and enables rapid multi-compound screening within a single chromatographic procedure.

The chromatographic conditions were therefore optimized to improve the separation of benzophenone derivatives while maintaining the good performance obtained for the original target analytes. The resulting method provides a versatile and efficient HPTLC approach for the simultaneous screening of UV photoinitiators and UV filters.

Standard solutions

The extended panel of analytes included: Irgacure 369 (CAS 119313-12-1), Irgacure 2959 (CAS 106797-53-9), benzophenone (CAS 119-61-9), 4-methylbenzophenone (CAS 134-84-9), TPO (CAS 75980-60-8), TPO-L (CAS 84434-11-7), BAPO (CAS 162881-26-7), 2-hydroxybenzophenone (CAS 117-99-7), 2,4-dihydroxybenzophenone (CAS 131-56-6), benzophenone-3 (CAS 131-57-1), benzophenone-4 (CAS 4065-45-6), and benzophenone-8 (CAS 131-53-3).

Each standard was dissolved in 0.2% triethylamine in acetonitrile and diluted to final concentrations of 0.5 mg/mL for Irgacure 369, Irgacure 2959, TPO, TPO-L, BAPO and benzophenone-8; 0.3 mg/mL for benzophenone-3 and benzophenone-4; 0.25 mg/mL for 2-hydroxybenzophenone and 2,4-dihydroxybenzophenone; and 0.125 mg/mL for benzophenone and 4-methylbenzophenone.

System Suitability Test (SST): Universal HPTLC Mix (UHM) prepared in-house [3], or commercially available (Sigma 91816).

[Note]: Amber glassware was used for standard preparation.

Sample preparation

Sample solutions were prepared by dissolving 50 mg of nail polish sample in 5.0 mL of 0.2% triethylamine in acetonitrile.

Material Analysis

Chromatogram layer

HPTLC glass plates silica gel 60 F_{254} (Supelco) 20 × 10 cm.

Sample application

Samples were applied as bands with a length of 8 mm, with the first application position at X = 20 mm and Y = 8 mm. The distance between tracks (center to center) is not less than 11.4 mm. Typical application volumes for samples were 4.0 µL, while UHM was applied at a volume of 2.0 µL.

Chromatography

Plates were developed using the ADC 2 according to the following protocol:

The plate was first developed to 50 mm with toluene, ethyl acetate, and methanol 8:1:2 (*V/V/V*) after activation at 33% relative humidity. After drying for 5 min in the ADC 2, the plate was subjected to a second development to 85 mm using toluene containing 0.1% acetic acid, again after activation at 33% relative humidity, followed by drying for 5 min in the ADC 2.

Documentation

The developed plates were documented using the TLC Visualizer 2 at 254 nm (detection A).

Densitometry

Absorption spectra were recorded with the TLC Scanner 4 from 190 to 900 nm using the deuterium and tungsten lamps, a slit dimension of 5.0 × 0.2 mm, and a scanning speed of 100 mm/s (detection B).

Scanning densitometry was performed in absorption mode using TLC scanner 4 at 224 nm (for TPO and TPO-L), at 214 nm (for BAPO), at 270 nm (for Irgacure 369, Irgacure 2959, benzophenone, 4-methylbenzophenone, and 2-hydroxybenzophenone), at 292 nm (for 2,4-dihydroxybenzophenone, benzophenone-3, benzophenone-4 and benzophenone-8), slit dimension 5.0 x 0.2 mm, scanning speed 20 mm/s (detection C).

Results and discussion

Evaluation of the previous HPTLC method

The HPTLC method previously established for the detection of the photoinitiators TPO, TPO-L, and BAPO was re-evaluated and extended to assess its applicability to additional benzophenone derivatives (Figure 1). The previously established method used ethyl acetate and toluene 2:8 (*V/V*) as developing solvent, with plate activation at 33% relative humidity for 10 min and a developing distance of 70 mm in the ADC 2.

Adequate separation was achieved for Irgacure 369 (track 7), 2,4-dihydroxybenzophenone (track 6), and benzophenone-8 (track 9), alongside the target photoinitiators TPO, TPO-L, and BAPO (tracks 4, 5, and 8, respectively). In contrast, benzophenone-3 (track 10), benzophenone (track 11), 4-methylbenzophenone (track 12), and 2-hydroxybenzophenone (track 13) displayed comparable R_F values and migrated close to the solvent front, resulting in insufficient chromatographic resolution for proper identification. Benzophenone-4 (track 2) remained at the application position, indicating strong retention on the stationary phase, whereas Irgacure 2959 (track 3) showed limited migration and was detected in the lower third of the plate.

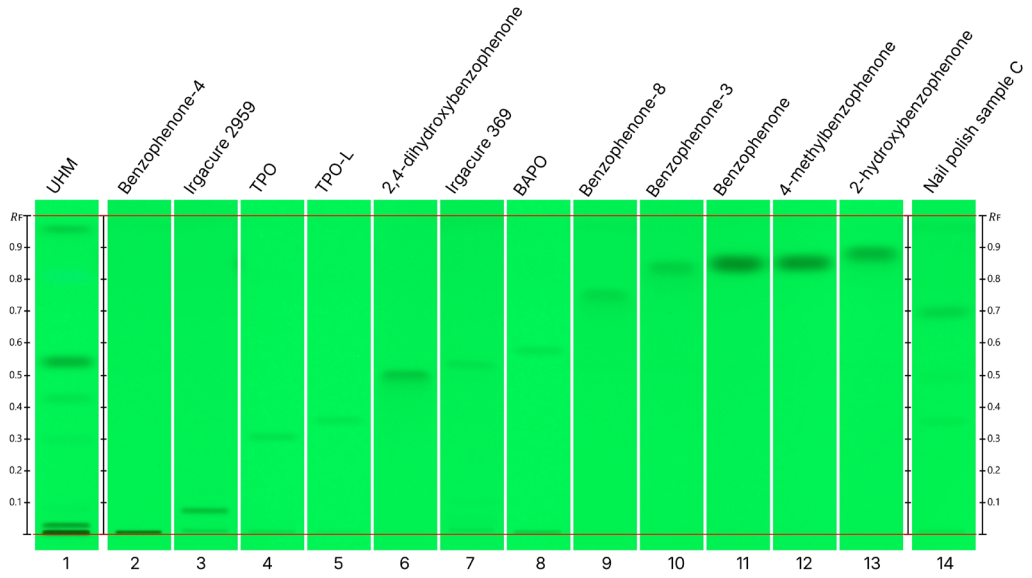


Figure 1: HPTLC fingerprints in shortwave UV light using the previously established HPTLC method for detection of TPO, TPO-L and BAPO.

Material Analysis

Method development

To achieve adequate separation of the benzophenone derivatives, the chromatographic conditions were systematically optimized. Particular attention was paid to maintaining the chromatographic performance previously established for the original target analytes. The initial experiments focused on improving the migration of benzophenone-4. As a first approach, the polarity of the developing solvent was increased (from 2.8 to 2.9), and the selectivity was modified by adding methanol (toluene, ethyl acetate, methanol 8:1:1 (*V/V/V*)). This modification induced slight migration of benzophenone-4 above the application position. However, the benzophenone derivatives in tracks 8–13 migrated toward the solvent front, with benzophenone and 4-methylbenzophenone showing nearly identical R_F values (Figure 2, tracks 10 and 11).

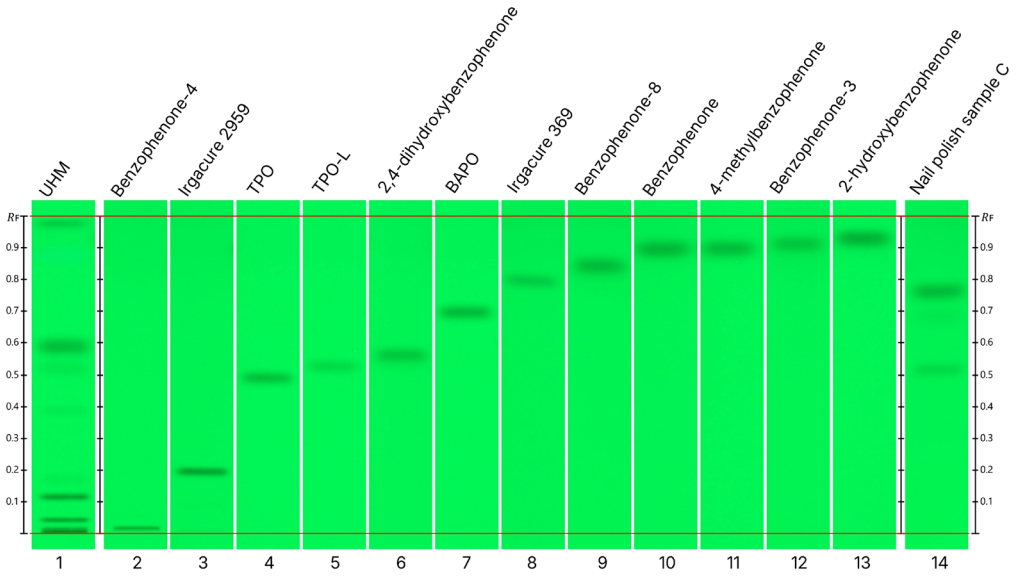


Figure 2: HPTLC fingerprints in shortwave UV light. Method development 1.

Because benzophenone-4 still showed limited migration, the methanol content of the developing solvent was further increased. The developing solvent consisted of toluene, ethyl acetate, methanol 8:1:2 (*V/V/V*). This modification improved the migration of benzophenone-4, resulting in an R_F value of 0.086 (Figure 3, track 2). In contrast, separation of the other benzophenone derivatives (tracks 8–13) remained insufficient; the analytes migrated into the upper third of the plate and exhibited similar R_F values.

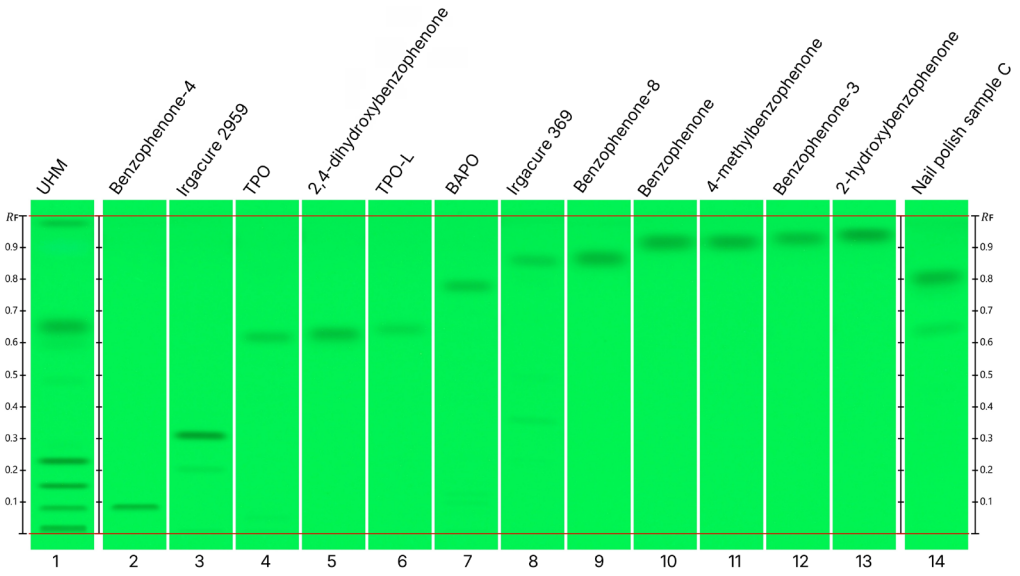


Figure 3: HPTLC fingerprints in shortwave UV light. Method development 2.

To better understand the migration behavior of the full analyte panel, chromatographic development was carried out using toluene as the sole developing solvent. Under these conditions, the photoinitiators, benzophenone-4, 2,4-dihydroxybenzophenone, and both Irgacure compounds (Figure 4, tracks 2–8) exhibited little to no migration from the application position. In contrast, the benzophenone derivatives in tracks 9–13 were adequately separated at distinct R_F values. These results demonstrated that toluene provided suitable selectivity for the less polar benzophenone derivatives but insufficient elution strength for the more strongly retained analytes.

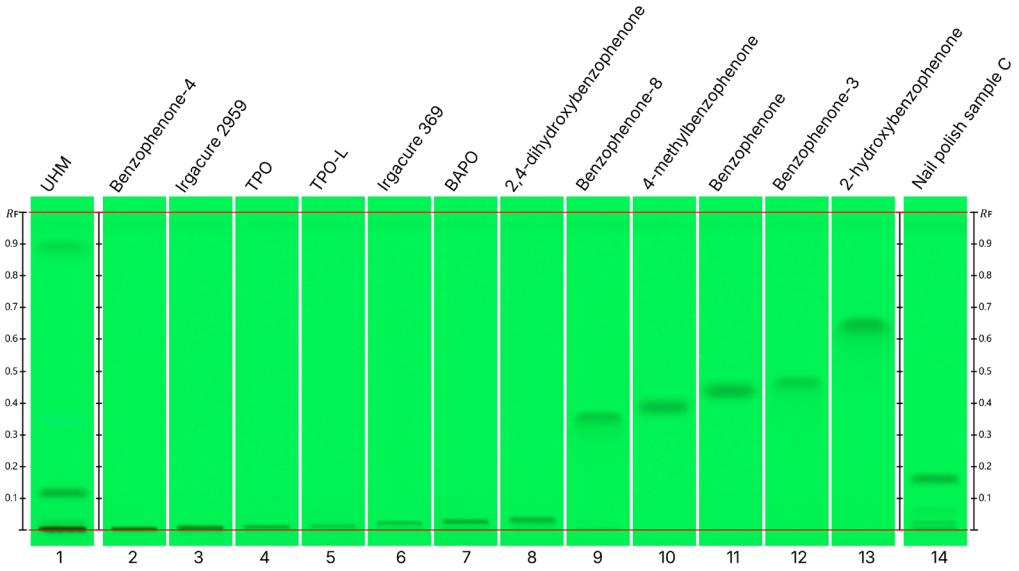


Figure 4: HPTLC plate in shortwave UV light. Method development 3.

To enhance the migration of benzophenone-4 without significantly influencing the migration of the other benzophenone derivatives, the proportion of toluene was decreased, resulting in a developing solvent composition of toluene, ethyl acetate, and methanol 6:1:2 (*V/V/V*). Under these conditions, the migration of benzophenone-4 was markedly improved. However, the modified developing solvent was unfavorable for the separation of the photoinitiators, as TPO, TPO-L, and 2,4-dihydroxybenzophenone exhibited similar R_F values and were insufficiently resolved (Figure 5, track 4–6). Furthermore, the remaining benzophenone derivatives migrated close to the solvent front, resulting in inadequate differentiation (tracks 8–13). Consequently, this composition did not provide satisfactory overall resolution of the target analytes.

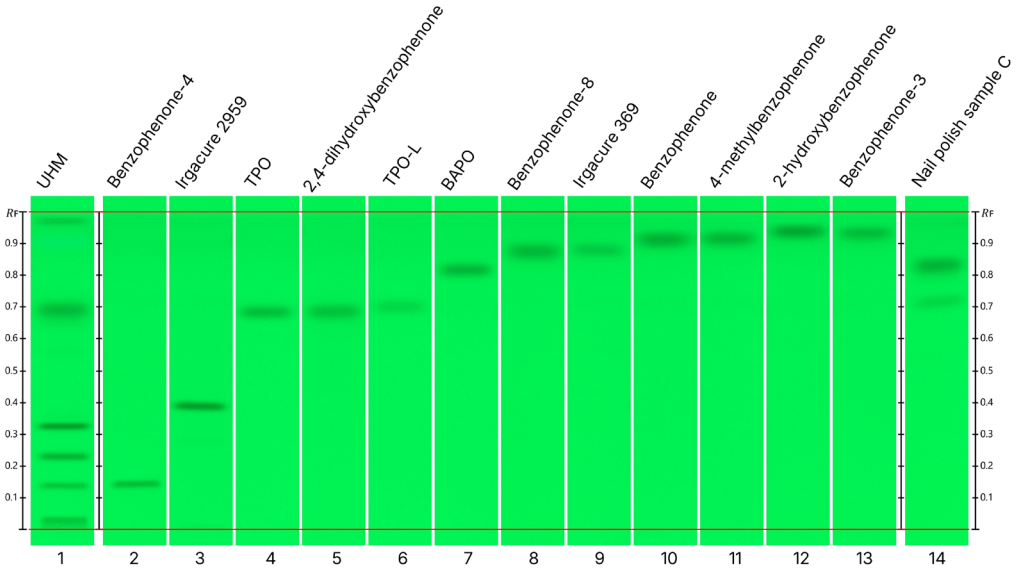


Figure 5: HPTLC plate in shortwave UV light. Method development 4.

Final HPTLC method

Because of the wide range of chromatographic behavior exhibited by the twelve analytes, satisfactory separation could not be achieved using a single developing solvent. A two-step development was therefore established based on the observations obtained during method optimization. The first development, using toluene, ethyl acetate, methanol 8:1:2 (*V/V/V*) to a migration distance of 50 mm, primarily controlled the migration of the more strongly retained analytes, including benzophenone-4, Irgacure 2959, and the phosphine oxide photoinitiators (Figure 6, A).

The second development, using toluene containing 0.1% acetic acid to a migration distance of 85 mm, provided additional selectivity for the less polar benzophenone derivatives (Figure 6 B; tracks 9–13).

The resulting chromatogram provided improved differentiation of the analyte panel. TPO and TPO-L exhibited closely spaced migration positions under the unified screening conditions.

When chromatographic differentiation between these two photoinitiators is specifically required, the previously established HPTLC method dedicated to TPO, TPO-L, and BAPO may be used [2]. In the investigated nail polish sample, a photoinitiator zone with a migration position consistent with TPO-L was observed. The sample was commercially acquired in France and manufactured in the Netherlands, with TPO-L explicitly listed in the ingredient declaration. The analytical result obtained using the new method was therefore consistent with the declared composition, providing a proof-of-concept application of the method to a nail polish sample. The R_F values determined for the reference substances are summarized in Table 1. Figure 7 illustrates the densitometric profiles of the twelve reference substances recorded at their selected detection wavelengths (detection C).

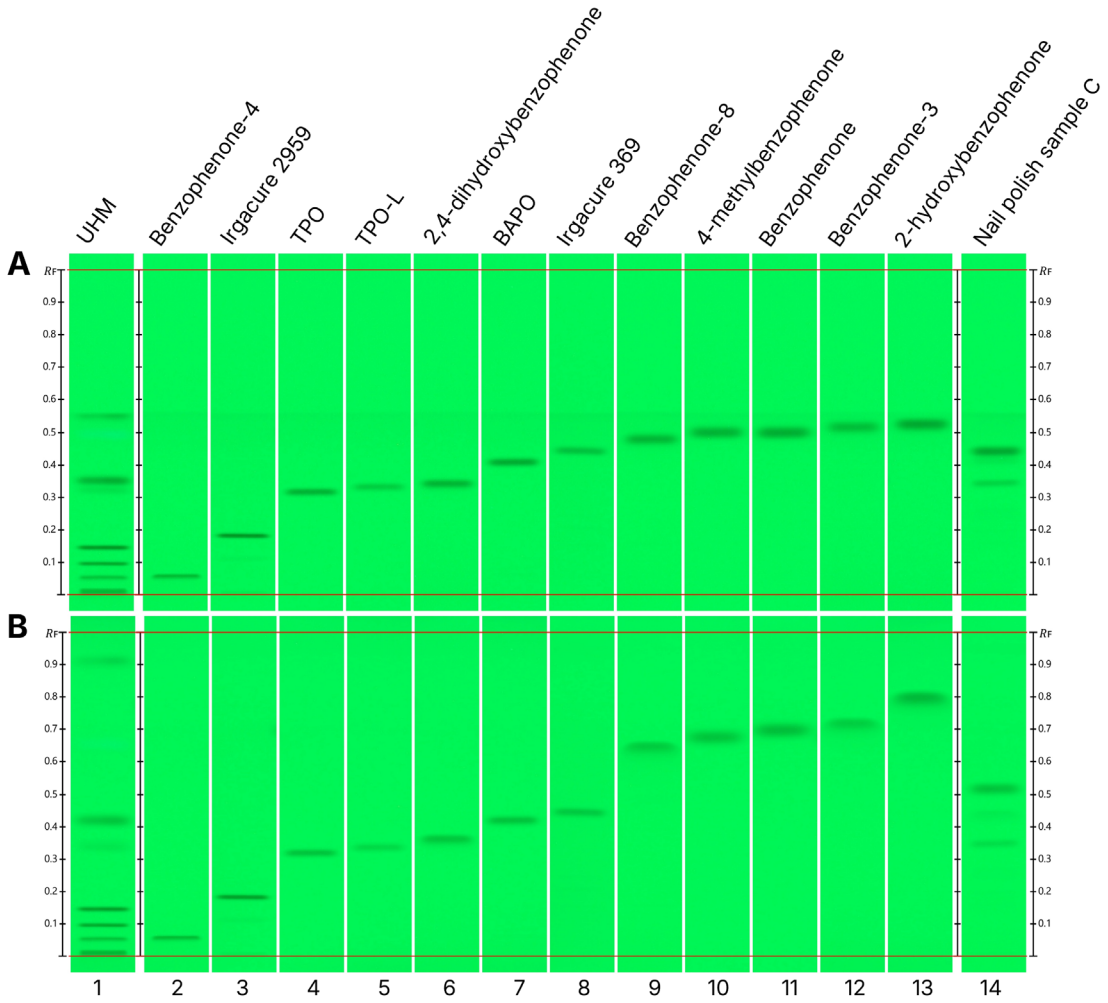


*Figure 6: HPTLC plate in shortwave UV light (detection A). **A**: first development 50 mm migration distance. **B**: second development 85 mm migration distance. Final method.*

Material Analysis

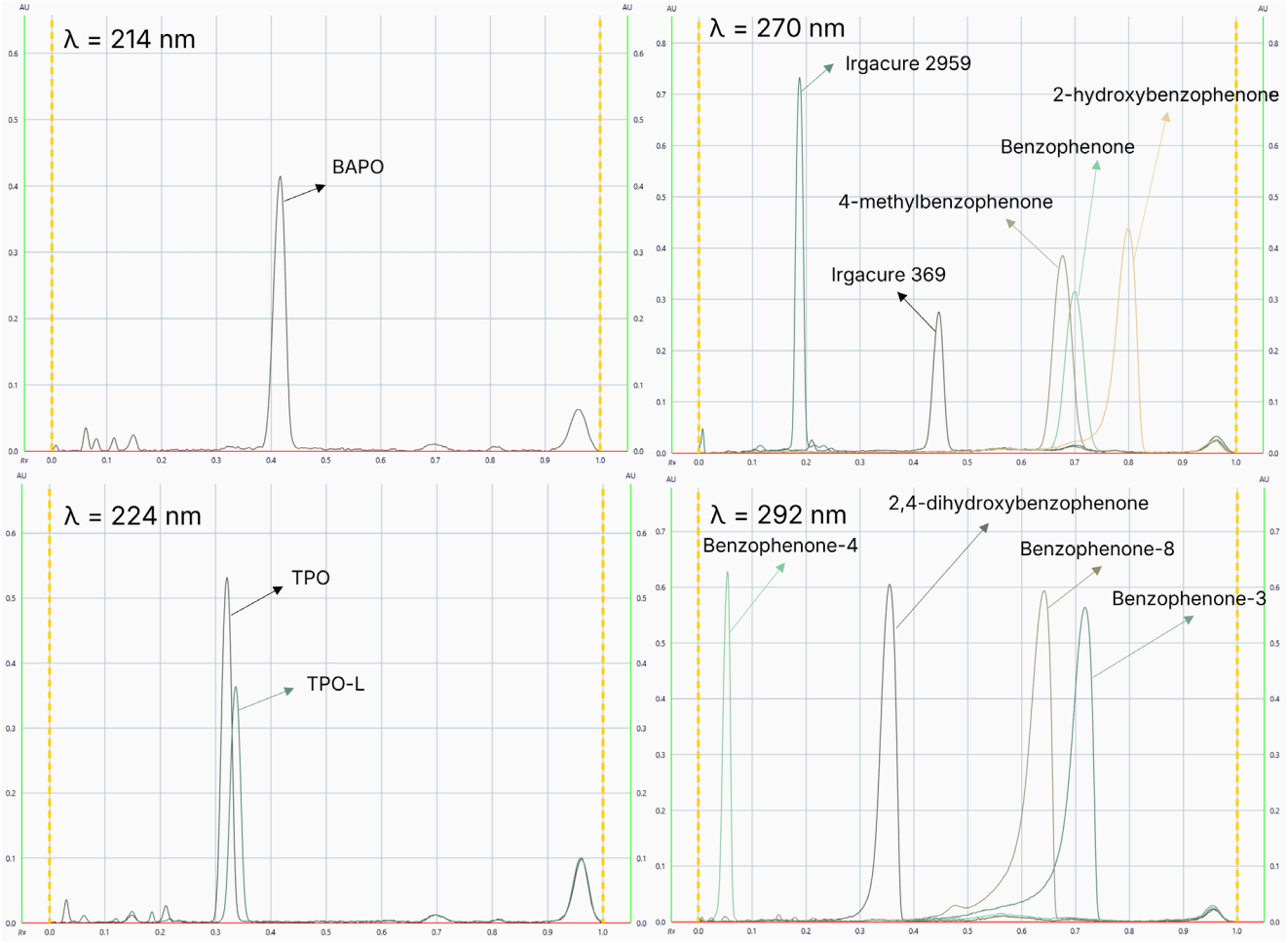


Figure 7: Peak profiles from scanning densitometry of the reference substances recorded at 214 nm (BAPO), 224 nm (TPO and TPO-L), 270 nm (Irgacure 2959, Irgacure 369, 4-methylbenzophenone, benzophenone, and 2-hydroxybenzophenone), and 292 nm (benzophenone-3, benzophenone-4, benzophenone-8, and 2,4-dihydroxybenzophenone) (detection C).

The R_F values of the twelve analytes showed low variability, with CV values ranging from 0.81% to 2.22% over five independent runs.

Table 1: R_F values of final method measured over 5 plates.

Track	Substance	R_F (n=5)	R_F CV [%] (n=5)
2	Benzophenone-4	0.054	1.96
3	Irgacure 2959	0.184	1.90
4	TPO	0.316	1.67
5	TPO-L	0.332	1.26
6	2,4-dihydroxybenzophenone	0.356	1.03
7	BAPO	0.415	0.86
8	Irgacure 369	0.444	2.22
9	Benzophenone-8	0.643	1.07
10	4-methylbenzophenone	0.673	0.86
11	Benzophenone	0.695	0.88
12	Benzophenone-3	0.715	0.81
13	2-hydroxybenzophenone	0.793	0.95

Material Analysis

System Suitability Test (SST)

The performance of the chromatographic procedure was evaluated over five independent runs using the Universal HPTLC Mix (UHM) as the system suitability test. The characteristic quenching zones showed consistent migration across the five plates, confirming the suitability and consistency of the two-step development procedure (Figure 8).

UHM (SST, Detection A); quenching zones (n=5) at:

- R_F ~ 0.053 ± 0.004
- R_F ~ 0.097 ± 0.003
- R_F ~ 0.147 ± 0.003
- R_F ~ 0.333 ± 0.005
- R_F ~ 0.413 ± 0.005

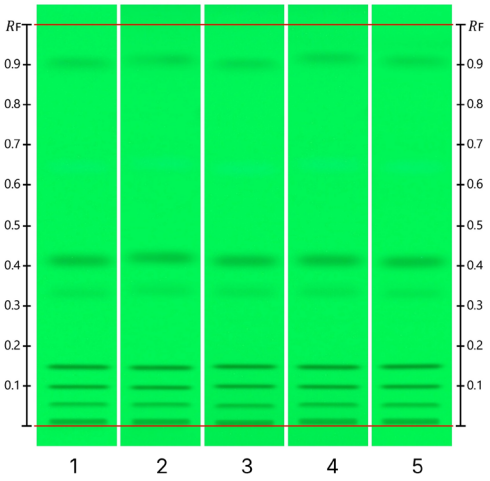


Figure 8: UHM (SST) in shortwave UV light

UV Spectral Characterization of TPO and TPO-L

Under the unified screening conditions, TPO and TPO-L cannot be reliably distinguished by migration behavior alone. However, their UV/Vis absorption spectra show distinct spectral profiles and therefore provide an additional means of differentiation. The UV/Vis spectra were acquired using a TLC Scanner 4 over a wavelength range of 190–900 nm (detection B). Although both compounds absorb in the region around 300 nm, TPO-L shows a more pronounced spectral feature in this region. The spectral profile obtained for sample C was much more comparable to that of the TPO-L reference standard than to that of TPO, supporting the assignment of the chromatographic zone to TPO-L (Figure 9).

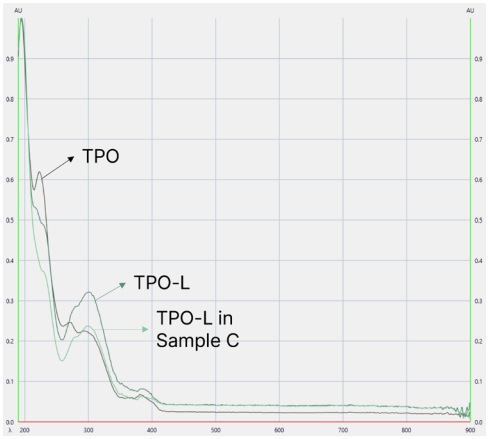


Figure 9: Spectrum densitometry of TPO, TPO-L and sample C (detection B).

Conclusion

A unified HPTLC screening procedure was developed for the analysis of twelve UV photoinitiators and benzophenone derivatives covering a wide range of chromatographic behavior. The two-step development combines complementary selectivities and provides improved differentiation of the target analytes compared with the previously established method. Consistent migration was observed over five independent runs, with R_F CV values ranging from 0.81% to 2.22%. For compounds exhibiting similar migration behavior, such as TPO and TPO-L, UV spectral characterization provided additional selectivity and supported their differentiation. The procedure was successfully applied to the screening of a commercial nail polish sample, demonstrating the potential of HPTLC for rapid multi-analyte screening of UV photoinitiators and UV filters.

Literature

[1] Aparicio *et al.*, Packag. Technol. Sci. 2015; 28: 181.

[2] CAMAG Application note A-150.1: HPTLC analysis of photoinitiators TPO, TPO-L, and BAPO in UV nail polish formulations.

[3] T. K. T. Do *et al.*, J Chrom. A (2021) 1638

Further information is available on request from the author(s).

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