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ORIGINAL RESEARCH PAPER

Development and validation of a high-performance thin-layer chromatography densitometric method for the simultaneous determination of novel 1-acridinyl-1,2,3-triazole derivatives

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Abstract
High-performance thin-layer chromatographic (HPTLC) method provides a simple, sensitive, and accurate analytical method for the simultaneous determination of certain synthesized 1-acridinyl-1,2,3-triazole derivatives without interference from starting materials and intermediates. Separation was carried out on Merck HPTLC silica gel $60F_{254}$ plates, using chloroform−methanol (9:1, *V/V*) and hexane−ethyl acetate (3:2, *V/V*) as mobile phases. Validation of the method was performed based on the basis of the International Council for Harmonisation (ICH) guidelines in terms of linearity, sensitivity, limit of detection, limit of quantification, precision, selectivity, and specificity. Least-square equations were calculated for the studied compounds in the ranges of 25–500 and 10–500 ng/spot for ultraviolet (UV) and fluorescence measurements, respectively. Correlation coefficients (r) values were found ranging from 0.9913 to 0.9992 for analytes. The method provides selectivity and specificity which ensure that synthesized compounds are in the pure form without the interference of starting materials and intermediates. The detection limits for the studied compounds ranged from 11.02 to 51.09 ng/spot and 3.84 to 31.95 ng/spot and quantification limits were 33.39–154.82 ng/spot and 11.63–73.67 ng/spot for both spectrophotometric and spectrofluorimetric methods, respectively, indicating applicability for good qualitative and quantitative determination of members of this series at the nanogram concentration levels in biological fluids.

Keywords 9-Azidoacridine · 1,2,3-Triazole · High-performance thin-layer chromatography (HPTLC) · Spectrophotometry · Spectrofluorimetry

1 Introduction

Thin-layer chromatography (TLC) is one of the most widely used techniques for separation, identification, and determination of drugs. It is an ideal technique because of its simplicity, low costs, selectivity, sensitivity, and ability to be performed without a remote area with limited volumes of solvents [1–3]. Cancer is one of the most common diseases worldwide, and there is an urgent need for more effective anticancer agents. The effectiveness of heterocyclic, tricyclic, and planar arrays to intercalate between base pairs in the double-stranded deoxyribonucleic acid (DNA) structure [4] and the ability of acceptor/donor nitrogen are two major considerations for the use of acridines as potential anticancer compounds. Additionally, acridines are characteristically highly crystalline, stable, attractively colored, and strongly fluorescent in nature. Acridine derivatives are characterized by unique physical and chemical properties, biological activities, and industrial applications [5–7]. Among these are their anti-inflammatory, anticancer, antimicrobial, antitubercular, antiparasitic, antimalarial, antiviral and fungicidal activities. Furthermore, acridines are used as fluorescent materials for visualization of biomolecules and in laser technologies. These properties of acridines are attributed to their semi-planar heterocyclic rigid structures. In the present study, we attempted the development of a sensitive, precise, and accurate TLC densitometric method for separation and determination of the newly synthesized compounds **6–10** [8]. The suggested TLC densitometric

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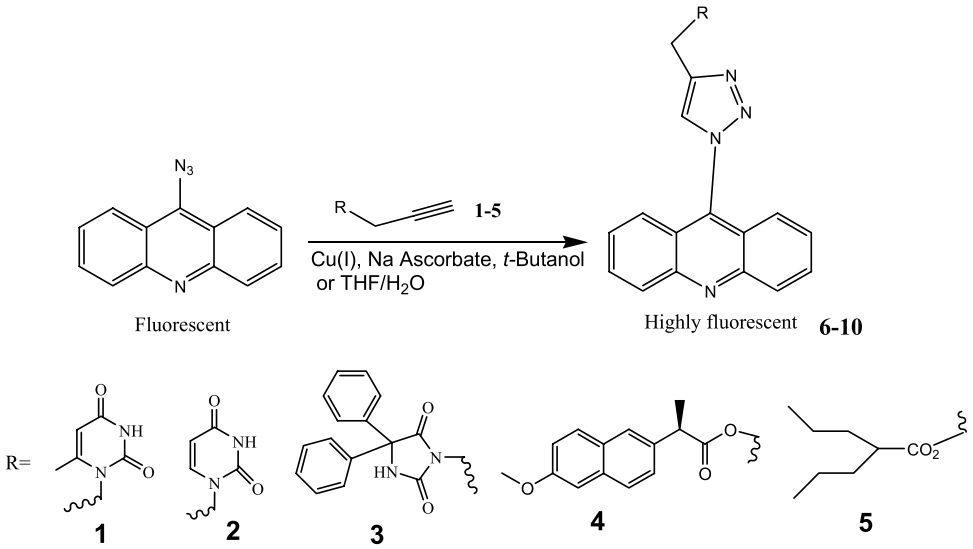
method will be designed to be time and costs saving and suitable for quality control work and validation procedures [9]. Our research proposal is based on the design of new hybridized pharmacophoric subunits in the molecular structure that maintain potential anticancer activity. Ligation of acridine via the triazole ring with nucleobase analogs uracil and 6-Me-uracil, deemed rationale for synthesis of cross-linkable DNA duplex [10]. Other groups, phenytoin [11] and valproic acid [12], are pharmaceuticals already in use as antiepileptics, while the (S)-naproxen [13] prescribed as non-steroidal anti-inflammatory drug (NSAID) is ligated as well to the 4th position of the triazole ring. Multiple pharmacological investigations of the three pharmaceuticals have uncovered the different mechanisms of their anti-tumor activity; however, the definite results were not yet upgraded to recommend their repositioning. But from an analytical view, we found that extended electron conjugation of the triazole might enhance fluorescence of acridine ring which provides useful analytical methods for labeling the synthesized derivatives as presented by Scheme 1 and Fig. 1 [14]. We found that 9-azidoacridine (having intrinsic fluorescent properties) upon reaction with the non-fluorescent propargyl features R (1–5) yielded the highly fluorescent 1-(acridin-9-yl)-4-substituted-1,2,3-triazoles. Based on this strategy, linking acridine nucleus to nonfluorescent molecule through 1,2,3-triazole ring under mild reaction conditions, we were able to develop the high-performance thin-layer chromatographic (HPTLC) densitometric method using fluorescence technique as rapid, sensitive tool for detection of the named biologically active anticancer and topoisomerase IIB inhibitors.

2 Experimental

2.1 Instrumentation

A CAMAG (Muttenz, Switzerland) TLC Scanner 4 was used for scanning by absorbance and fluorescence, wavelength 190–900 nm complete with deuterium lamp, tungsten-halogen lamp, and mercury vapor lamp. Scanning speed is selectable up to 20 mm s^{-1} while data resolution can be selected to 100 µm/step. Data acquisition was done by use of winCATS software, version 1.4.10 (CAMAG). CAMAG Linomat 5, 230 V was used for sample application spot- or bandwise in quantitative and qualitative TLC. 100 µL Hamilton (Bonaduz, Switzerland) glass sample syringe was used for dosing of samples. CAMAG twin-trough chamber was used for plates 20 × 10 and 10 × 10 cm with stainless steel lid.



Scheme 1 Copper(I)-catalyzed 1,3-dipolar cycloaddition reaction of fluorescent 9-azidoacridine and terminal alkynes (**1–5**) afforded intense fluorescent 1,2,3-triazole products

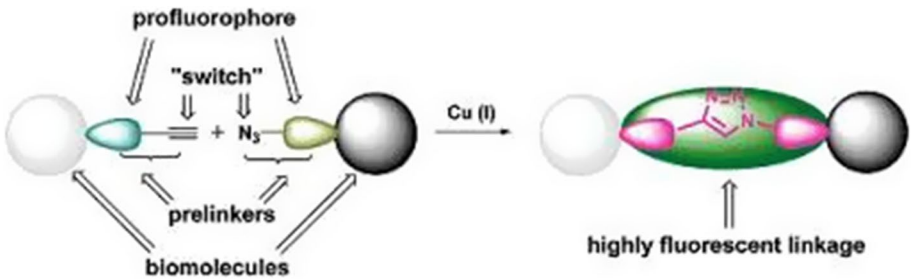


Fig. 1 Schematic illustration of the "clicking-and-probing ligation." Prelinkers containing azide or alkyne moieties which upon cycloaddition would lead to formation of triazolyl units with enhanced fluorescent emissions [14]

2.2 Materials and reagents

All solvents and reagents used were of analytical reagent grade. High-performance liquid chromatography (HPLC) grade methanol was obtained from Sigma-Aldrich (Steinheim, Germany). Chloroform, hexane, and ethyl acetate were obtained from Fluka Co. (Buchs, Germany). Pure compounds **6–10** were synthesized by the general procedure as given in Scheme 1. Data of the structure homogeneity of the studied compounds **6–10** were confirmed by ^{1}H- and ^{13}C-NMR (nuclear magnetic resonance), element analysis, and mass spectrometry (MS), cited in [7] by the same author. HPTLC aluminum plates pre-coated with silica gel 60 F_{254} (20 × 20 cm, 6–8 µm particle sizes, 250 µm thickness) were obtained from Merck (Darmstadt, Germany).

2.3 Preparation of sample solutions

2.3.1 Stock solutions

An accurately weighed amount of 10 mg of any of the studied compounds **6–10** was transferred into 100 mL volumetric flask and dissolved in about 50 mL of methanol. The mixture was shaken for 5 min then sonicated for 5–10 min. The solution was then completed to the volume with methanol to obtain a stock solution containing 100 µg mL^{-1}.

2.3.2 Working solutions

Working solutions were prepared by further dilution of suitable volumes (0.2–10 mL) of stock solutions with methanol in separated 10 mL volumetric flasks to obtain concentrations ranging from 2 to 100 µg mL^{-1} corresponding to 10–500 ng/spot for ultraviolet (UV) and fluorescence measurements according to the ranges indicated in Table 1. (In all cases, 5 µL volumes as 4 mm bands were applied.)

Table 1 Different concentration ranges of the studied compounds used for both UV and fluorescence measurements

Compound	UV measurements (ng/spot)	Fluorescence measurements (ng/spot)
6	50–330	10–110
7	30–330	20–110
8	120–420	20–140
9	75–500	75–500
10	25–500	10–200

2.4 Chromatographic conditions

A volume of 10 mL of the mobile phase chloroform–methanol (9:1, *V/V*) for compounds **6–8**, hexane–ethyl acetate (3:2, *V/V*) for compounds **9–10** were poured into the TLC tank which was lined with thick filter paper to help saturation of the tank. Then the tank was covered with a lid and pre-saturated with the vapors of the mobile phase system for 30 min at room temperature before use. The samples were spotted in the form of bands of width 4 mm with CAMAG 100 µL sample syringe (Hamilton) on HPTLC silica gel 60 F_{254} (E. Merck) using CAMAG Linomat 5. A constant application rate of 0.1 µL s^{-1} was employed and space between two bands was 1.0 cm. The application volume was 5 µL; sample solutions were spotted on the marked edge of the TLC plate 1 cm apart from the lower edge of the plate. The slit dimension was kept at 5 × 0.45 mm. The sample-loaded HPTLC plate was transferred to TLC tank and the plate then developed until a migration distance of 80 mm from the origin was reached. The plate was removed, air-dried for about 5 min, and viewed under UV lamp. The TLC plate was then measured using TLC scanner in the reflectance–absorbance mode at 254 nm for compounds **6–10** for all measurements and measured using fluorescence mode at 362 nm for compounds **6–9** and 350 nm for compound **10**. All measurements were operated by winCATS software (CAMAG). Concentrations of the compounds chromatographed were determined from the intensity of diffusely reflected light indicated by the peak areas in the resulting chromatograms. The produced peak areas were correlated to analyte concentrations using the regression package saved to the winCATS software and Excel 2016 (Microsoft Corporation, Redmond, WA, USA) for each target compound.

3 Results and discussion

3.1 Synthesis of targeted 1-acridinyl-1,2,3-triazole derivatives as anticancer agents

The targeted 1-acridinyl-1,2,3-triazole derivatives **6–10** were synthesized via a 1,3-dipolar cycloaddition reaction of 9-azidoacridine with the propargyl small molecules (R = 1–5) under click reaction conditions [7] as indicated in Scheme 1.

3.2 Biological evaluation

3.2.1 In vitro cytotoxicity activity of the target compounds

The hybrid compounds **6–10** were screened by the National Cancer Institute (NCI) (Bethesda, MD, USA) in a primary anticancer assay in the full NCI-60 cell panel. The cell lines were derived from nine different cancer types: melanoma, leukemia, colon, lung, central nervous system, renal, ovarian, breast, and prostate cancers. Compounds **9** and **10** having naproxen and valproic acid fragments showed low cell growth inhibition against all cancer cell lines. However, compounds **6–8** having 6-methyluracil, uracil, and phenytoin fragments displayed cell growth inhibition and selectivity against the different cell lines. Compound **6** displayed good to excellent cell growth inhibition against five types of cancer cell lines: leukemia (K-562, HL-60 [TB], and SR), melanoma (LOX IMVI), ovarian (IGROV1), prostate (DU-145), and breast (MCF7) with percent growth inhibition of 83.7%, 92.9%, 88.6%, 84.8%, 67.0%, 84.3%, and 97.5%, respectively. For compound **7**, cell growth inhibition ranging from 61.3 to 67.0% was observed against K-562 and SR, MCF-7 and T-47D cell lines. Compound **8** showed comparable cell growth inhibition percent ranging from 60.8 to 68.6% against K-562, SR, and MDA-MB-468; however, it showed marked selectivity and the best inhibitory activity of 84.1% against NCI H522 (non-small cell lung cancer) [7].

3.2.2 MTT assay for cell viability

The cytotoxicity of compounds **6–8** against MCF-7 breast cancer and DU-145 prostate cancer cell lines was determined using the MTT assay. The compounds were all more cytotoxic in the MCF-7 cell line and in both cell lines the 6-methyuracil derivative **6** was more cytotoxic than the uracil derivative **7** with the bulk phenytoin derivative **8** the least active (Table 2). Compound **6** displayed comparable cytotoxicity (IC50 2.70 ± 0.08 mM) with the standard doxorubicin (IC50 2.06 ± 0.04 mM), an anthracycline in clinical use as a wide spectrum antitumor agent, which exerts its action by intercalation between DNA double strands and blocks the topoisomerase IIB involved in the DNA replication process [7].

3.2.3 Topoisomerase IIB assay

The acridine ligands **6–8** were evaluated for inhibitory activity against MCF7 topoisomerase IIB, and compounds **6** and **7** showed potent inhibitory activity with IC_{50} of 0.52 mM and 0.86 mM, respectively, compared to doxorubicin IC_{50} 0.83 mM, while the phenytoin derivative **8** was less active with IC_{50} 2.56 mM (Table 2). Cell-cycle analysis of MCF7 and DU-145 cells treated with the active ligands **6–8** exhibited cell-cycle arrest at G2/M phase and proapoptotic activity [7].

3.3 Spectral analysis

Absorption spectra of the studied compounds were investigated in the general range from 200 to 400 nm. The maximum absorption wavelengths were found 254 nm for the studied compounds. Excitation wavelength was determined at the λ_{max} 254 nm while the emission wavelengths were 362 nm for compounds **6–9**, and 350 nm for **10** as shown in Table 3 and Fig. 2 for the starting agent 9-azidoacridine, and the triazoloacridinyl compounds **7–9** as representative examples. In the current work, complete resolution was obtained by using HPTLC plates, and each member of the studied compounds can be quantified at its specified R_F value and using the specified wavelengths.

3.4 Mobile phase composition and saturation time

Due to the marked difference in polarities and functional groups for the studied drugs, we cannot use a single mobile phase for their separation. Thus, a mixture of solvents was used for separation of the studied compounds with different ratios of solvent components. Compact spots as well as good separation with reasonable R_F values were obtained for each of the studied compounds by using the mobile

Table 2 Cytotoxicity profile and topoisomerase IIB inhibitory activity of compounds **6–8**

Compound	Cytotoxicity IC_{50} (μM ± SE)		Topoisomerase IIB
	MCF7	DU-145	IC_{50} (μM)
6	2.70 (±0.08)	26.10 (±1.25)	0.52
7	10.86 (±0.61)	59.34 (±2.61)	0.86
8	42.71 (±1.54)	79.27 (±1.84)	2.56
Doxorubicin	2.06 (±0.04)	14.28 (±0.51)	0.83

Table 3 Assigned wavelengths for UV and fluorescence measurements

Compound	UV measurement (λ_{max})	Fluorescence measurement	
		(λ_{ex})	(λ_{em})
9-Azidoacridine	254	254	362
6	254	254	362
7	254	254	362
8	254	254	362
9	254	254	362
10	254	254	350

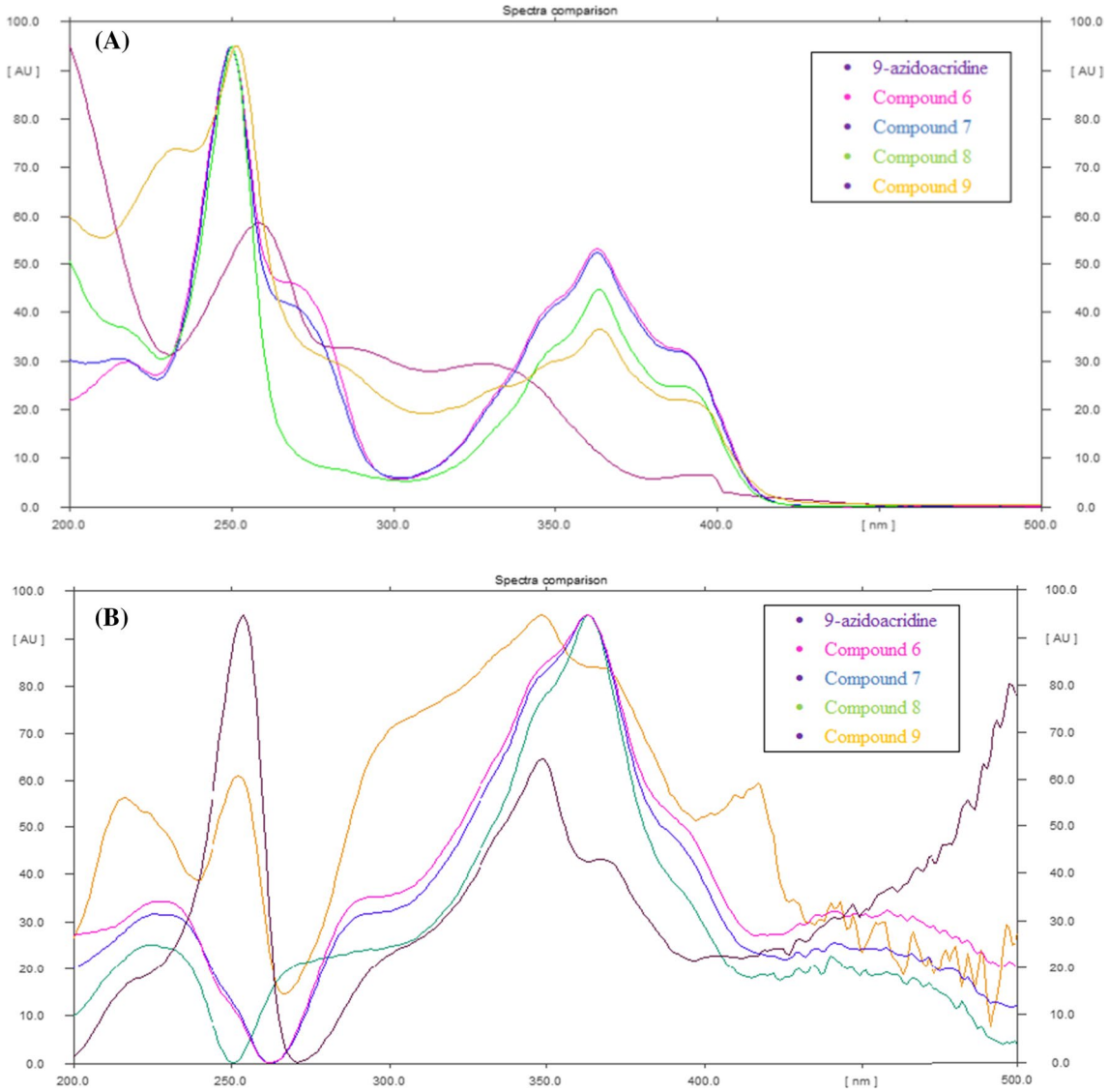


Fig. 2 Absorption (**A**) and emission (**B**) spectra of compounds 9-azidoacridine, **6–9** in the range of 200−500 nm

Table 4 Solvent systems used for separation of the studied compounds

Compound	Mobile phase	Ratio (*V/V*)	R_F
9-Azidoacridine	Chloroform−methanol	9: 1	0.88
6	Chloroform−methanol	9: 1	0.38
7	Chloroform−methanol	9: 1	0.29
8	Chloroform−methanol	9: 1	0.46
9	Hexane−ethyl acetate	3: 2	0.36
10	Hexane−ethyl acetate	3: 2	0.65

phases indicated in Table 4 and Fig. 3 as representing example. Several trials were made by changing both the polarity and composition of different solvents. Chloroform or hexane was selected as one of the components of the mobile phase with acceptable resolution. However, the R_F value was too small, so the solvent strength was increased by adding a polar solvent. Methanol was added to chloroform, while ethyl acetate was added to hexane to develop improved chromatograms. The mobile phase comprising chloroform−methanol (8:2, *V/V*) showed good resolution. Saturation time ranging from 15 min up to 45 min was tried in order to obtain compact good separated spots for all

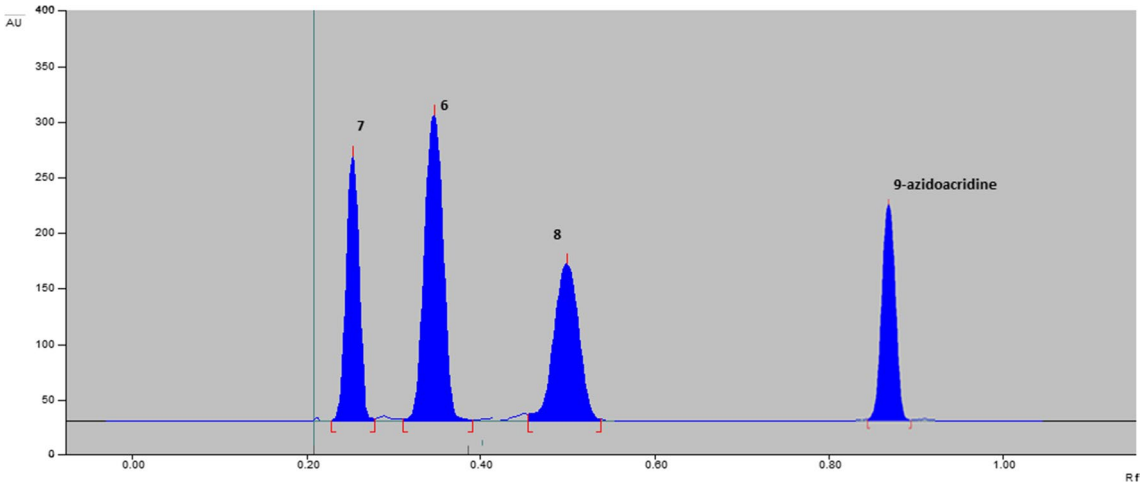


Fig. 3 Two-dimensional TLC densitogram for compounds 9-azidoacridine, **6, 7,** and **8** at concentration level of 600 ng/band for all compounds and using mobile phase (chloroform−methanol, 9:1, *V/V*)

investigated compounds. It was found that the highest optical intensities and good resolution were obtained at saturation time between 25 and 30 min. A 30 min saturation time was selected for all compounds in the subsequent work.

3.5 Validation of the HPTLC method

The method was validated according to the International Council for Harmonisation (ICH) (QR2) guidelines and also complied with the United States Pharmacopeia (USP) 31-NF 26 for the determination of range, linearity, limits of detection and quantification, precision, selectivity, and specificity. For most of the calculations and statistical analysis, Excel 2016 was used.

3.5.1 Calibration range and linearity

According to the ICH guidelines, the range of analytical procedure is the interval between the upper and lower concentration of an analyte in the sample for which it has been demonstrated with a suitable level of precision, accuracy, and linearity, while linearity of an analytical procedure is its ability (within a given range) to obtain test results that are directly proportional to the concentration of analyte in the studied sample. Six concentration levels or more (three replicates for each) were selected to construct the calibration curves. The selected concentrations in the range from 10 to 500 ng/spot, the assigned wavelengths λ_{max} 254 nm for the UV, and the wavelengths λ_{ex} 254, λ_{em} 362, 350 nm for the fluorescence measurements were indicated for each compound (Table 5). Linear regression data revealed good linear relationships between the concentrations used and the corresponding peak areas with correlation coefficients ranging from 0.9913 to 0.9992. Figures 4, 5, 6, 7, 8, 9 10, 11, 12, and 13 show the linear calibration curves and the three-dimensional densitograms for calibration levels of the studied compounds **6–10**.

Table 5 Statistical parameters of the regression equations

Comp. No	Linearity range (ng/spot)		Correlation coefficient (*r*)		Intercept ± SD (a)		Slope ± SD (b)		Standard deviation of residuals	
	UV	Fluor	UV	Fluor	UV	Fluor	UV	Fluor	UV	Fluor
6	50−330	10−110	0.9964	0.9992	1154.15 ± 84.79	641.69 ± 55.71	20.97 ± 0.634	102.57 ± 1.853	131.16	169.54
7	30−330	20−110	0.9968	0.9991	501.51 ± 59.1	844.90 ± 39.45	14.71 ± 0.308	77.36 ± 2.758	63.77	252.37
8	120–420	20−140	0.9913	0.9976	2076.5 ± 230.6	904.89 ± 75.27	14.90 ± 0.987	139.89 ± 4.81	247.74	402.64
9	75−500	75−500	0.9991	0.9988	1875.06 ± 99.5	1212.31 ± 90.97	15.55 ± 0.322	14.48 ± 0.348	111.06	120.21
10	100−500	10−200	0.9933	0.9992	458.73 ± 175.92	355.89 ± 97.59	18.24 ± 0.713	95.98 ± 3.54	230.21	160.81

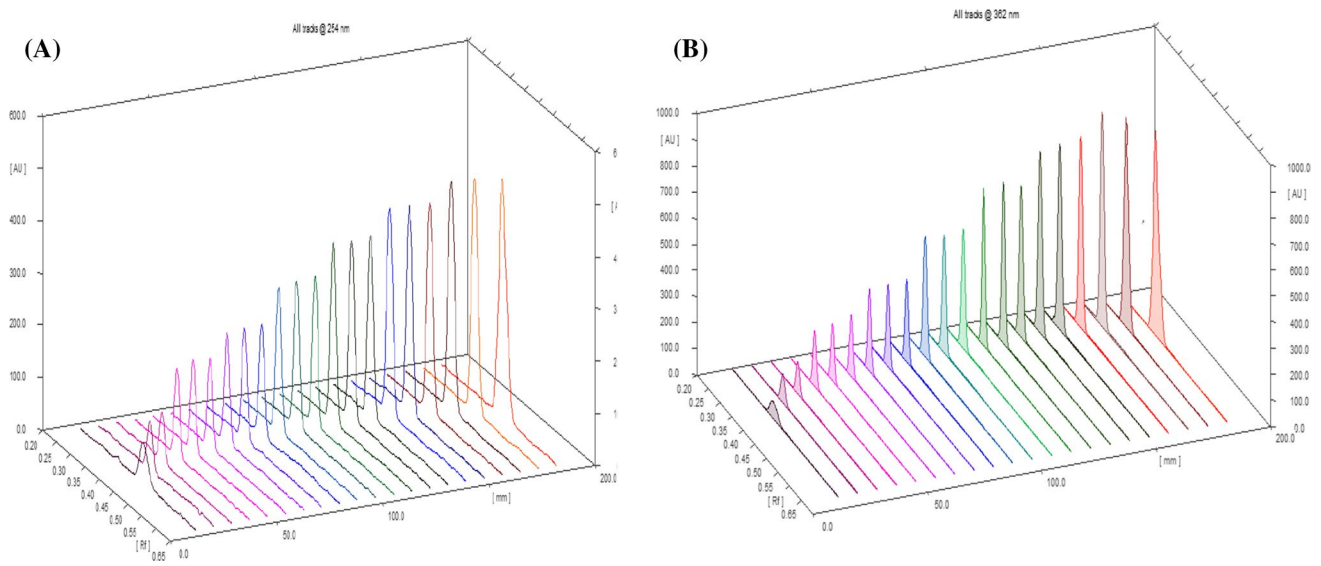


Fig. 4 Three-dimensional HPTLC densitograms of the calibration ranges of compound **6** using UV (**A**) and fluorescence (**B**) measurements

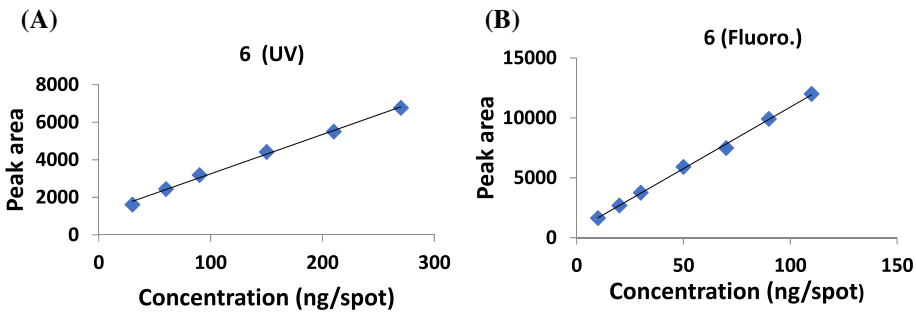


Fig. 5 The linear calibration curves for the compound **6** measured spectrophotometrically (**A**) and spectrofluorometrically (**B**)

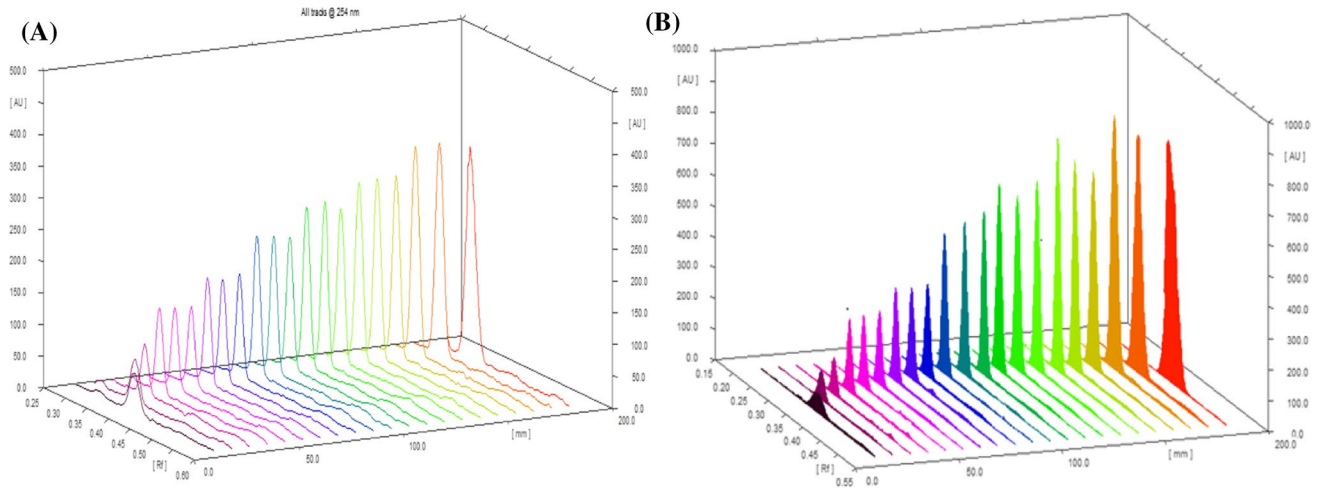


Fig. 6 Three-dimensional HPTLC densitograms of the calibration ranges of compound **7** using UV (**A**) and fluorescence (**B**) measurements

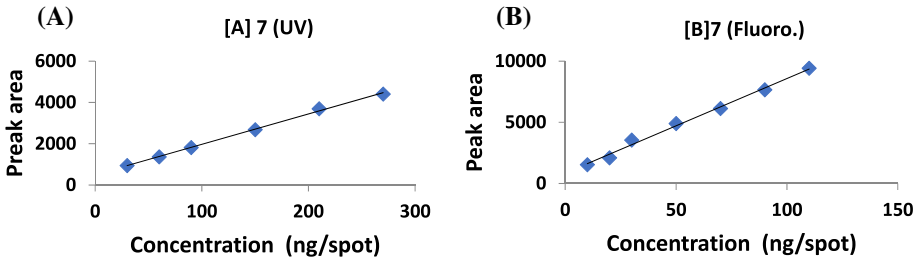


Fig. 7 The linear calibration curves for the compound **7** measured spectrophotometrically (**A**) and spectrofluorometrically (**B**)

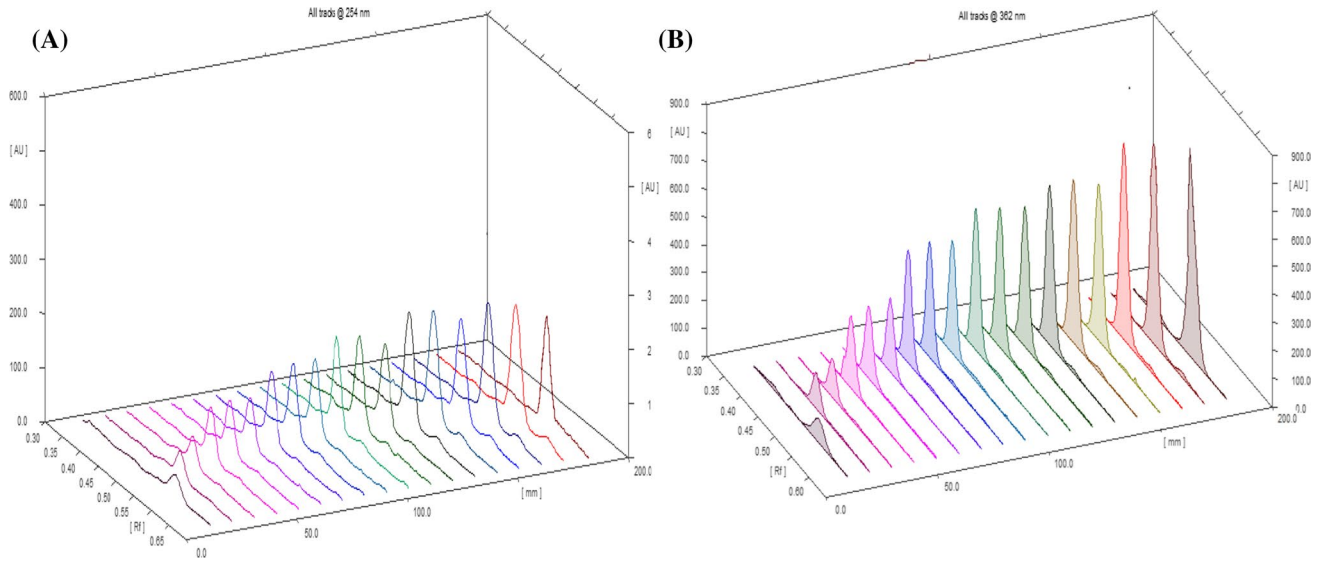


Fig. 8 Three-dimensional HPTLC densitograms of the calibration ranges of compound **8** using UV (**A**) and fluorescence (**B**) measurements

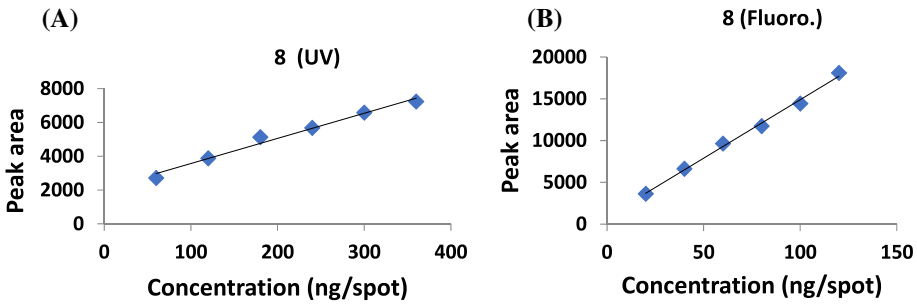


Fig. 9 The linear calibration curves for the compound **8** measured spectrophotometrically (**A**) and spectrofluorometrically (**B**)

3.5.2 Sensitivity

The sensitivity of the method was determined in terms of limit of detection (LOD) and limit of quantitation (LOQ). LOD and LOQ were determined for the investigated compounds using the formula:

$$\text{LOD or LOQ} = KSD_a/b,$$

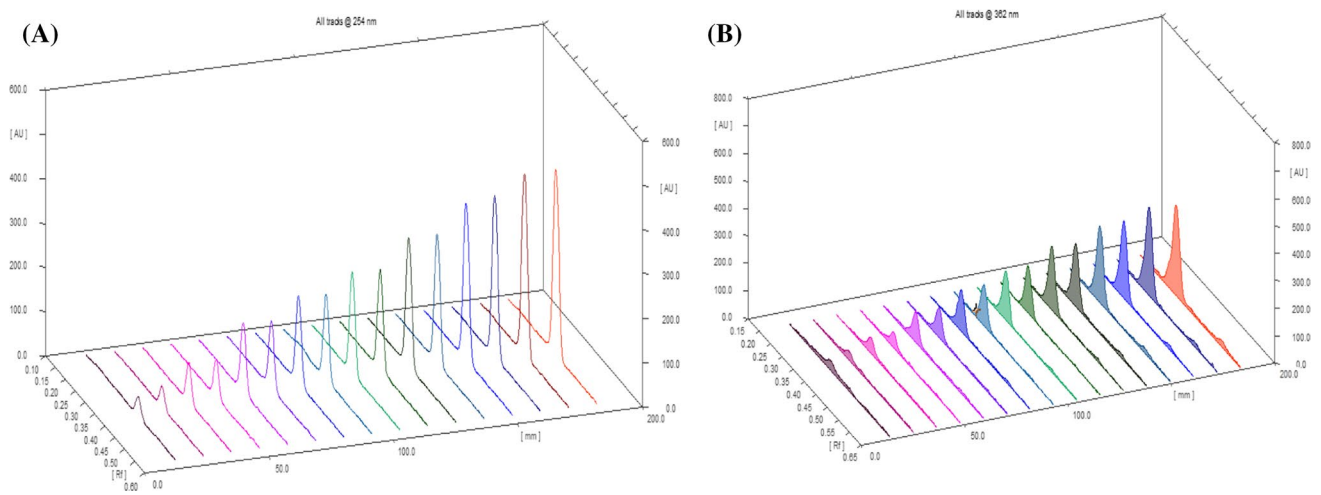


Fig. 10 Three-dimensional HPTLC densitograms of the calibration ranges of compound **9** using UV (**A**) and fluorescence (**B**) measurements

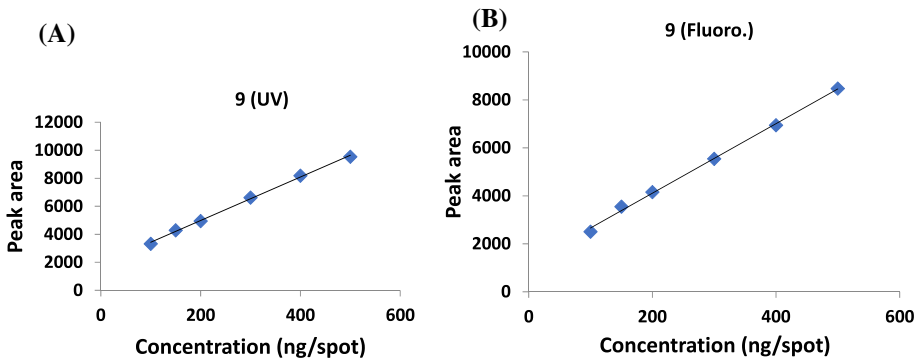


Fig. 11 The linear calibration curves for the compound **9** measured spectrophotometrically (**A**) and spectrofluorometrically (**B**)

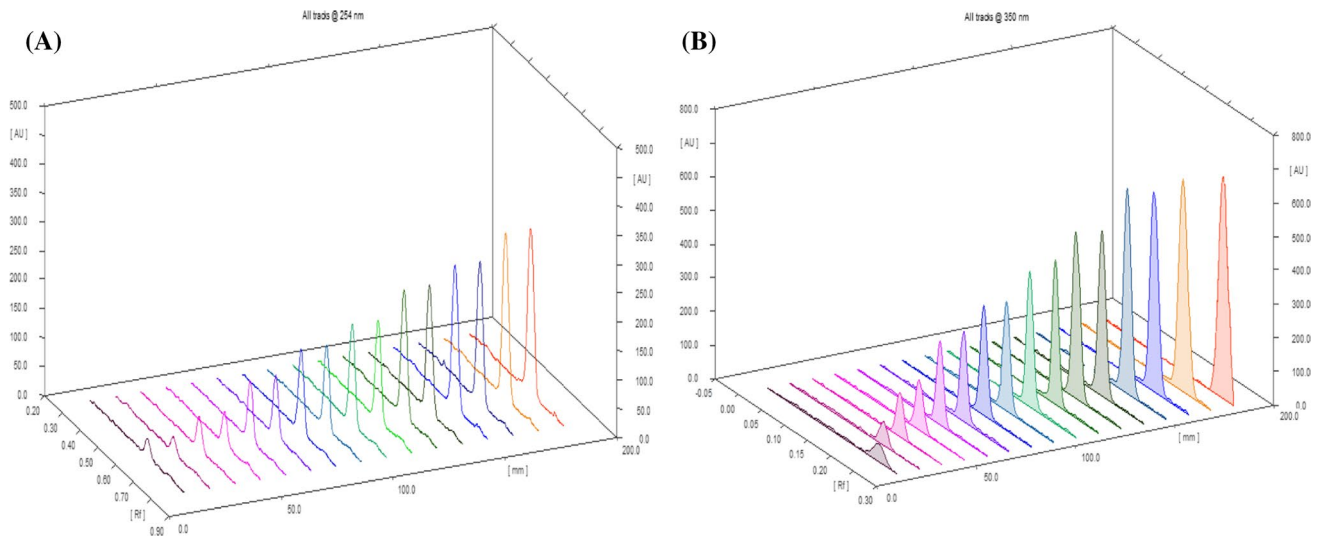


Fig. 12 Three-dimensional HPTLC densitograms of the calibration ranges of compound **10** using UV (**A**) and fluorescence (**B**) measurements

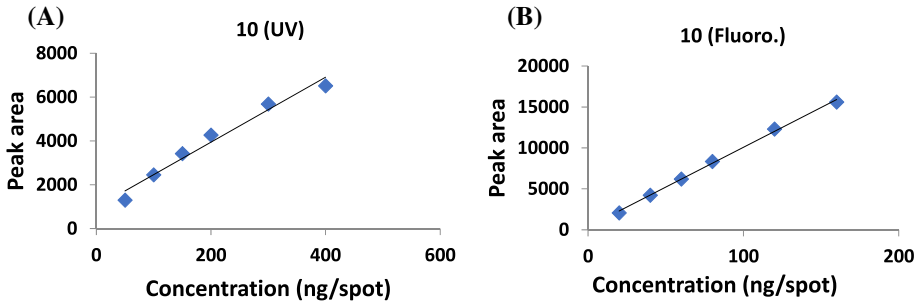


Fig. 13 The linear calibration curves for the compound **10** measured spectrophotometrically (**A**) and spectrofluorometrically (**B**)

Table 6 Linearity ranges, limits of detection, and quantitation for the studied compounds **6–10** determined by the proposed HPTLC method

Compound	Linearity range (ng/spot)		LOD (ng/spot)		LOQ (ng/spot)	
	UV	Fluor	UV	Fluor	UV	Fluor
6	50–330	10–110	15.89	3.84	48.15	11.63
7	30–330	20–110	11.02	7.57	33.39	22.95
8	120–420	20–140	51.09	8.84	154.82	26.80
9	75–500	75–500	21.13	24.55	63.39	73.67
10	100–500	10–200	31.83	5.88	96.45	17.82

where K is a constant = 3.3 for LOD and 10 for LOQ, SDa is the standard deviation of the blank determination or standard deviation of intercept of the calibration curve, and b is its slope. The LOD values of the synthesized compounds **6–10** were found to be 11.02–51.09 ng/spot and 3.84–31.95 ng/spot for both spectrophotometric and spectrofluorometric method, respectively. These were the least amounts of analyte in a sample that can be detected but not necessarily quantitated as an exact value. The LOQ values for the synthesized compounds **6–10** were 33.39–154.82 ng/spot and 11.63–73.67 ng/spot for both spectrophotometric and spectrofluorometric method, respectively. These were the lowest concentrations of the drugs that were accurately detected and integrated by the instrument used.

The calculated LOD and LOQ values for all compounds studied are shown in Table 6, indicating high sensitivity of the proposed methods. Upon comparison of LOD and LOQ values obtained by the proposed method with those of the reported method for doxorubicin, there was a significant difference: LOQ was 12.5 ng/mL for doxorubicin and 11.63 ng/mL for compound **6** and LOD was 5 ng/mL for doxorubicin and 3.84 ng/mL for compound **6** indicating high sensitivity [14].

3.5.3 Selectivity and specificity

Selectivity of analytical method is the measure of the extent to which the method can determine a particular compound in the analyzed matrices without interference from the sample matrix or degradation products. As indicated by the previous results for separation and determination of the studied compounds, there is no interference between them and the starting materials or intermediates peaks, and a good separation of their peaks was obtained in all cases. Figure 14 indicates the separation of compound **6** in the presence of their starting materials as representative example of the studied compounds. In addition, method selectivity was determined using peak purity test. The purity of the synthesized compounds was confirmed by ^{1}H- and ^{13}C-NMR spectroscopy, elemental analysis, and MS [7]. The peak purity of the studied compounds was assessed by comparing peaks of the studied compound and starting material at peak start, peak apex, and peak end position.

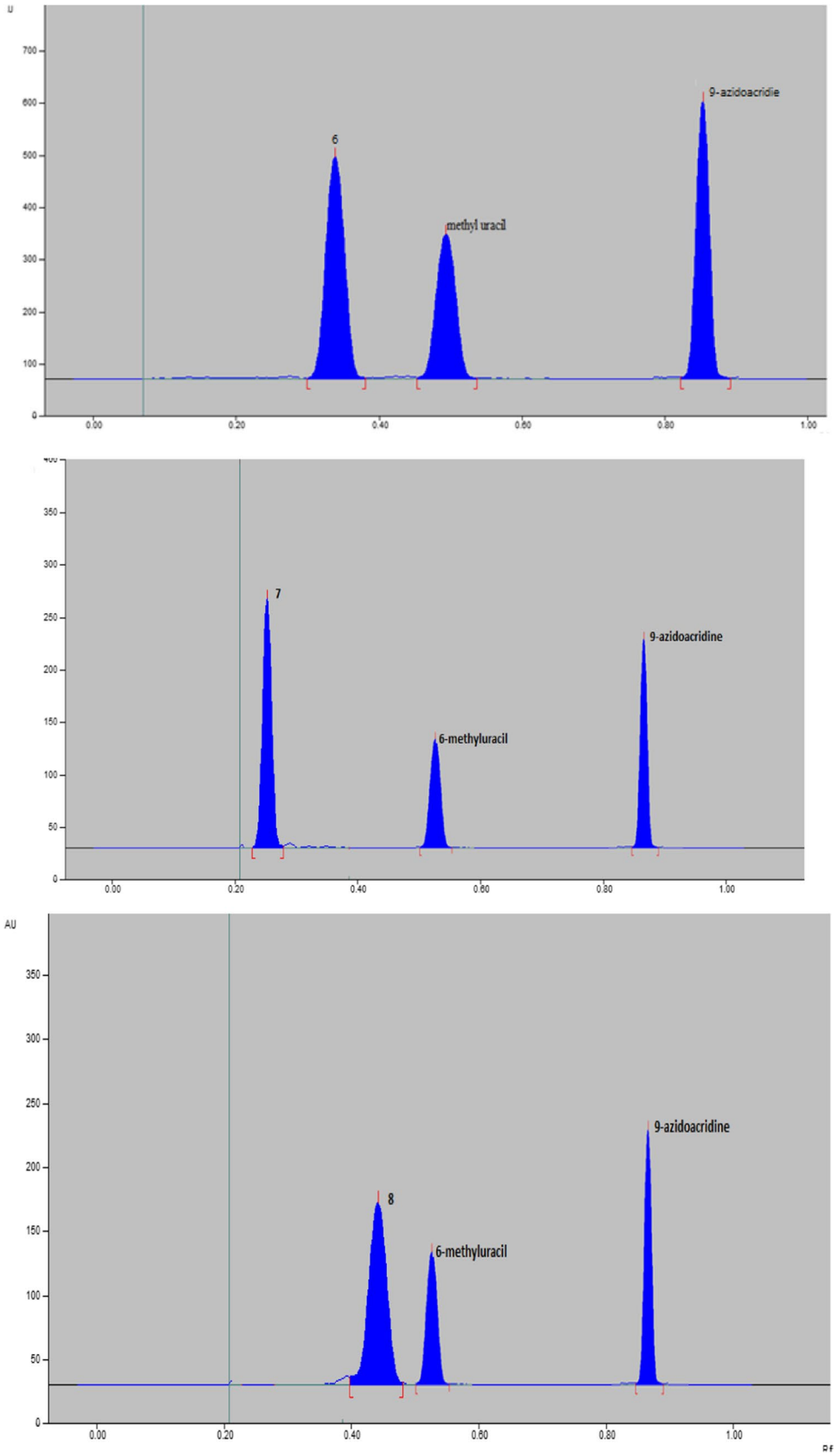


Fig. 14 Two-dimensional TLC densitogram of compounds **6–8** and starting material at concentration level of 600 ng/band for all compounds using the mobile phase chloroform−methanol (9:1, *V/V*)

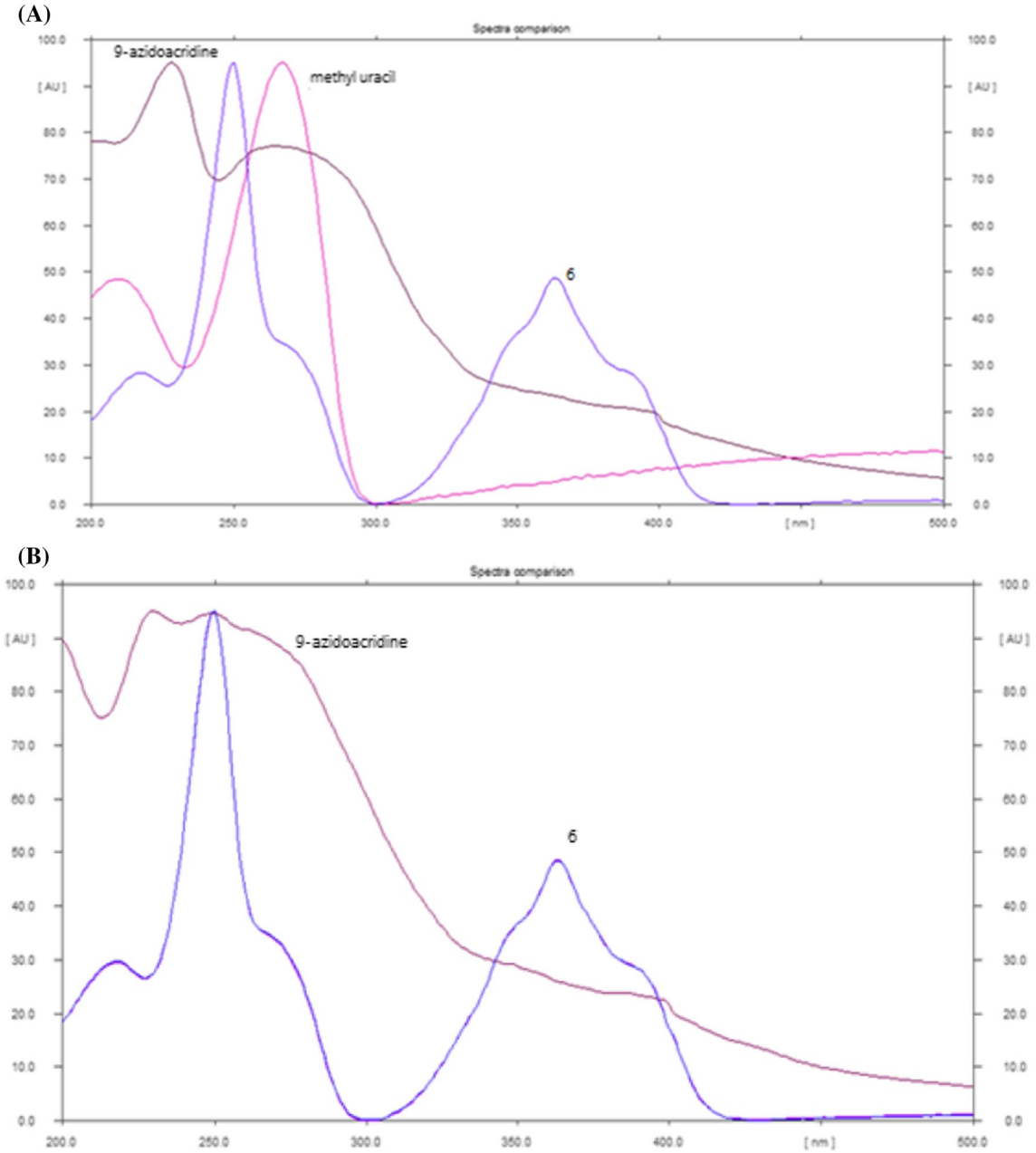


Fig. 15 Spectrum of compound **6** in comparison with spectra of starting materials as representative example of studied compounds with both UV (**A**) and fluorometric detection (**B**)

The results of comparison indicated the closeness in these positions in both studied compound and starting material. The bands for all compounds were confirmed by comparing spectra, as shown in Fig. 15, peak area, and R_F values of the sample spots. The method is considerably selective and suitable for the determination of the studied compounds in the pure form and in the presence of starting materials and intermediates.

3.5.4 Precision

The precision of the method was expressed as relative standard deviations (RSD). RSD% values ranged from 0.34% to 4.47% indicating good repeatability and precision as shown in Table 7. The obtained precisions were satisfactory for quality control measurement.

Table 7 Precision studies of the developed method

Comp. No	Drug concentration [ng per band]	RSD [%][a]	Drug concentration [ng per band]	RSD [%][a]
6	10	1.31	30	4.74
	50	0.69	150	2.27
	110	3.44	330	5.72
7	20	2.43	60	3.41
	70	2.84	210	0.61
	110	1.73	330	3.19
8	20	2.78	60	1.20
	80	1.19	180	1.57
	120	3.00	360	1.79
9	25	1.16	50	2.44
	150	2.66	200	0.34
	400	1.41	500	0.56
10	25	1.36	50	1.34
	150	3.20	200	2.34
	400	1.92	500	1.56

[a]Estimated from three determinations at each concentration level

4 Conclusion

The developed HPTLC method provides a simple, precise, and accurate analytical method for the simultaneous determination of the synthesized compounds **6–10**. The method was validated as per the ICH guidelines. The correlation coefficient (*r*) values ranging from 0.9913 to 0.9992 for analytes indicate that the method is suitable to quantify the studied drugs without any interference from intermediate and starting material. The selectivity, simplicity, precision, and sensitivity of the proposed TLC method allowed the application of the developed procedure for the analysis of target drugs in human plasma in the future. A simple fluorometric HPTLC method for the simultaneous monitoring of new 1,2,3-triazoloacridine derivatives has been developed. These compounds having compliance to linearity, precision, and high sensitivity can be recommended for the quantitative analysis of anticancer agents in pharmaceutical preparations. So, this study demonstrated that the characteristic fluorescence properties of the studied compounds made it possible to detect and/or measure this anticancer activity in biological systems via the use of fluorescence-based techniques. Application in biological systems, our study would help in the ADMET monitoring phase of drug development and would help understand the detailed mechanisms of action of these synthesized compounds as anticancer agents as well as the development of novel modalities to enhance their therapeutic index.

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Declarations

Conflict of interest The authors declare that they do not have any conflict of interest.

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