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Analysis of anticancer drugs using thin layer chromatography– A review

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ABSTRACT: Cancer is a fatal disease and cancer incidence is increasing from day to day. Thus, anticancer drugs are widely used and their analysis requires simple and effective analytical procedures. Thin layer chromatography (TLC) is a promising technique for drug analysis as a simple and versatile technique with low cost of analysis, minimal sample clean-up and high sample loading capacity. An extensive literature survey has been done and TLC techniques used for the analysis of anticancer agents in different matrices have been presented.

KEYWORDS: Thin layer chromatography; anticancer drugs; biological fluids; pharmaceuticals; plants.

1. INTRODUCTION

In cancer, cell growth can not be controlled causing tumor [1, 2]. Generally, chemotherapeutics are used as a first treatment choice. The chemotherapeutics can be grouped as antimetabolites, antitubulin drugs, DNA-interactive drugs, molecular targeting drugs, hormones, monoclonal antibodies and other biological agents [2]. The main classes and the most commonly used anticancer drugs, as given below, are discussed in this review.

- **Antimetabolites:** Purine analogues (6-mercaptopurine, 6-thioguanine, azathioprine, clofarabine, fludarabine) and pyrimidine analogues (5-fluorouracil, capecitabine, tegafur, cytarabine, 5-azacytidine, gemcitabine) belong to this class of anticancer drugs. Other antimetabolites are methotrexate, raltitrexed, pentostatin and hydroxycarbamide. Their mechanism of action is based on the interaction with essential biosynthesis pathways. Among this class, 5-fluorouracil is a widely used anticancer drug for the treatment of breast, gastrointestinal tract and certain skin cancers. Tegafur and capecitabine are metabolised to 5-fluorouracil and are given orally for metastatic colorectal cancer. Gemcitabine is a more recently introduced compound of the antimetabolites and is used intravenously in association with cisplatin for metastatic non-small cell lung, pancreatic and bladder cancers. Azathioprine, a purine analogue, is an antileukaemic drug and is metabolised to 6-mercaptopurine. Mercaptopurine is also directly used as a maintenance therapy for acute leukaemia. Chemical structures of selected antimetabolite drugs are given in Figure 1.
- **Antitubulin drugs:** This group interfere with microtubule dynamics, block division of the nucleus and lead to cell death. The main members of antitubulin drugs are vinca alkaloids (vindesine, vincristine, vinblastine, vinorelbine) and taxanes (docetaxel, paclitaxel). Vinca alkaloids have proven efficacy in treatment of certain solid tumours (mainly lung and breast), lymphomas and acute leukaemia. Taxanes are mainly used for the treatment of ovarian and breast cancer. They are also used for advanced non-small-cell lung cancer. Chemical structures of selected antitubulin drugs are given in Figure 2.

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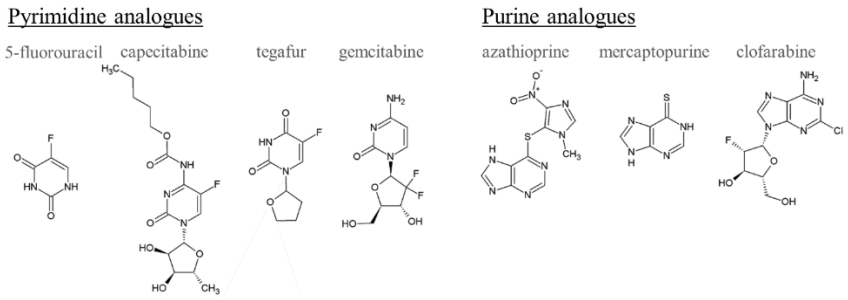

Figure 1. Chemical structures of selected antimetabolite drugs.

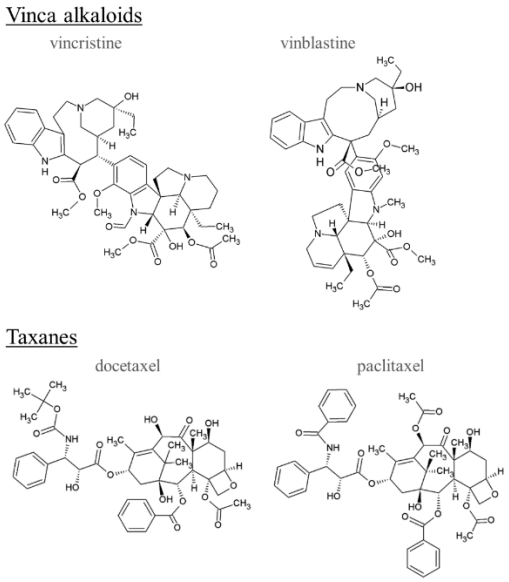

Figure 2. Chemical structures of selected antitubulin drugs.

- **DNA interactive drugs:** They have a variety of action mechanisms:
  Alkylating drugs (dacarbazine, temozolomide, procarbazine) lead to the alkylation of DNA bases.
  Cross-linking drugs including nitrogen mustards (cyclophosphamide, ifosfamide), platinum complexes (cisplatin, carboplatin, oxaliplatin) and other cross-linking drugs (thiotepa, busulfan, carmustine) function by binding to DNA resulting to an intra-strand or inter-strand cross-linking of DNA.
  Intercalating drugs act by binding between base pairs (e.g., doxorubicin, daunorubicin, aclarubicin, epirubicin)
  Topoisomerase inhibitors including topoisomerase I inhibitors (topotecan, irinotecan) and topoisomerase II inhibitors (teniposide, etoposide) inhibit the responsible enzymes for the cleavage, annealing and topological state of DNA.
  DNA-cleaving drugs such as bleomycin interact with DNA and cause strand scission at the binding site.
  Chemical structures of selected DNA interactive drugs are given in Figure 3.
- **Molecularly targeted drugs:** Kinase inhibitors (imatinib, trastuzumab) belong to this group of anticancer drugs.
- **Hormones:** Anti-estrogens (toremifen, raloxifen, tamoxifen) and aromatase inhibitors (anastrozole, aminoglutethimide) are used for the treatment of breast cancer. The other members of this group, gonadorelin analogs (leuprolide, buserelin) and anti-androgens (flutamide, bicalutamide) have a significant activity against prostate cell lines [2].

  Chemical structures of selected molecularly targeted drugs and hormones are given in Figure 4.

Thin layer chromatography (TLC) is a method in which test sample is applied to the chosen stationary phase, and the plate is developed with the mobile phase to allow the separation to occur. Then the plate is dried, and various methods can be applied to obtain detection, qualitative analysis, and quantification of the compound zones. All these steps are fully automated by use of available commercial instruments. Also,

analytical throughput and speed in TLC are high compared to other analytical methods because many samples can be chromatographed simultaneously. Moreover; HPTLC plates with smaller particle size sorbents and thinner layers offer faster, more efficient separations with better resolution than TLC plates.

The most prominent application areas of TLC include pharmaceutical products, plant materials, foods and beverages, environmental samples and radiochemical purity of labeled drugs [3, 4].

Methods for TLC and HPTLC (together termed planar chromatography) were reviewed many times by Sherma [4-7]. Generally these reviews include current practice of TLC, important advances in this area and a variety of TLC applications. The review of HPTLC methods for drug analysis was published in 2010 for the period of 1996-2009 [4].

Analytical techniques for the separation of anticancer agents were discussed in a previous review in which only a few HPTLC methods were mentioned [8].

In 2013, a book was published including TLC applications of all drug groups. In a chapter of this book, TLC of anticancer drugs was discussed by Yeniceli [9].

According to our knowledge, there is no recent paper including TLC systems used for the analysis of anticancer drugs in different matrices. In this review, an extensive literature survey has been done and many TLC methods used for the analysis of anticancer agents have been presented. Also, the use of TLC method for lipophilicity determination has been reported.

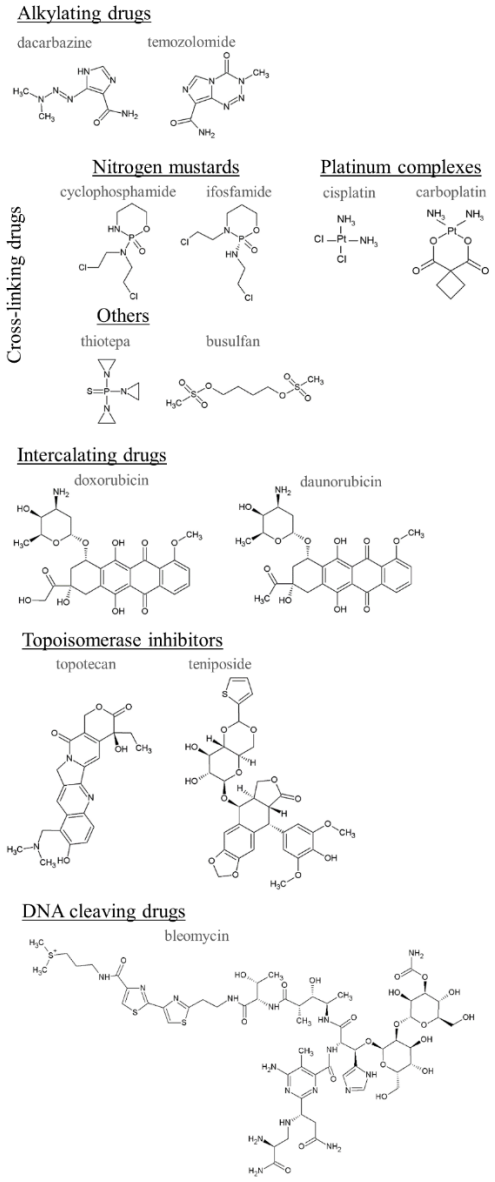

Figure 3. Chemical structures of selected DNA interactive drugs.

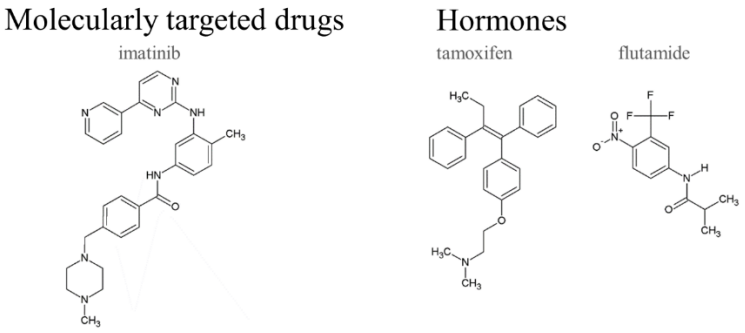

Figure 4. Chemical structures of selected molecularly targeted drugs and hormones.

2. ANALYSIS OF ANTICANCER DRUGS USING TLC METHOD IN BULK DRUG AND PHARMACEUTICALS

Bourget and his group reported many HPTLC methods for the determination of anticancer agents in capsules and infusion bags as a part of pharmaceutical quality control programme in a hospital chemotherapeutics developing unit [10-15]. Experimental procedures of these methods are given in Table 1.

In another study, the chromatographic behaviors of aclarubicin and doxycycline using different stationary and mobile phases were investigated [16]. Most of the published method development studies include stability indicating HPTLC of the anticancer agents in bulk drug and pharmaceuticals [17-22]. In one of these methods, Vadera et al. developed a stability indicating HPTLC method for the determination of imatinib mesylate as a bulk drug and in pharmaceuticals. After the treatment of acid, base, oxidation and heat; the drug undergoes degradation under all these conditions [17]. The stability indicating HPTLC methods of dasatinib, irinotecan, gemcitabine HCl, anastrozole and leuprolide acetate were also reported [18-22].

Apart from these stability indicating methods, Kulkarni et al. developed a simple and specific HPTLC method for the determination of azathioprine in pharmaceuticals [23]. In another study, Sharma and Sharma determined irinotecan HCl in pharmaceutical dosage forms using spectrophotometric and TLC methods [24]. HPTLC methods were also developed for the separation of tamoxifen citrate from dissolution media constituents and the analysis of bicalutamide in bulk drug and liposomes [25, 26]. Experimental procedures of these methods are given in Table 1.

Incorporation of anticancer drugs into liposomes allows their antitumor effect to be optimized. Saetern et al. investigated the stability of camptothecin containing liposomes by an HPTLC method [27]. In another study, the fabrication of thermosensitive liposomes used in combination with local hyperthermia (40 – 43°C) was evaluated in order to increase the selectivity of doxorubicin action and a TLC method was developed for the analysis of thermosensitive liposomes loaded with doxorubicin [28]. The combination of daunorubicin and 6-mercaptopurine in liposomes was also investigated for better chemotherapy and liposome stability experiments were evaluated by using TLC method [29].

Apart from these methods, there are several pharmacopoeia monographs of anticancer drugs including TLC methods. As an example, seven impurities of 5-fluorouracil were specified and the determination of two impurities (2-ethoxy-5-fluorouracil and urea) was reported in the European Pharmacopoeia. The separation was performed on a TLC silica gel F_{254} plate developed with methanol-water-ethyl acetate (15:15:70) [30]. In addition; 6-mercaptopurine, the degradation product of azathioprine, is limited by the British and American Pharmacopoeia to less than 1% (w/w) by TLC [31, 32].

Table 1. TLC Methods of Anticancer Drugs in Bulk Drug and Pharmaceuticals

Compounds	Sample	Adsorbent	Solvent System	Detection	Ref.
Fludarabine, cytarabine, gemcitabine, 5-fluorouracil	Infusion bags	TLC Silica gel	Ethyl acetate-methanol-water (50:10:10)	UV-270 nm	[10]
Vinca alkaloids	Infusion bags	TLC Silica gel 60 F_{254}	Dichloromethane-methanol (93:7)	Densitometry-274 nm	[11]
Busulfan	Capsules, infusion bags	TLC Silica gel 60 F_{254}	Ethyl acetate-chloroform- methanol (65:20:15)	4-nitrobenzyl pyridine (NBP) in ethanol	[12]
Cyclophosphamide	Capsules, infusion bags	TLC Silica gel 60 F_{254}	Dichloromethane-methanol-acetic acid (97:3:2)	1.25% phosphomolybdic acid in ethanol	[13]
Irinotecan and topotecan	Infusion bags	Nano-SIL®-20 UV_{254} plates	Methylene chloride-methanol-acetic acid-water (82:24:2:1)	Fluorescence ref. mode (exc. 366 nm, det. above 400 nm)	[14]
Imatinib mesylate	Bulk drug, pharmaceuticals	HPTLC Silica gel 60 F_{254}	Chloroform-methanol (6:4)	Densitometry-276 nm	[17]
Dasatinib	Bulk drug, pharmaceuticals	HPTLC Silica gel 60 F_{254}	Toluene-chloroform (7:3)	Densitometry-280 nm	[18]
Irinotecan	Bulk drug, injectables	TLC Silica gel 60F_{254}	Acetone-ethyl acetate - acetic acid (8.5:1.5:0.1)	UV-366 nm	[19]
Gemcitabine HCL	Pharmaceuticals	TLC Silica gel 60 F_{254}	Toluene-methanol-chloroform (3.6:3.6:3)	Densitometry-268 nm	[20]
Anastrozole	Bulk drug, tablets	TLC Silica gel	Toluene-acetone-ammonia (6:4:0.3)	UV-200 nm	[21]
Leuprolide acetate	Bulk drug	HPTLC Silica gel 60 F_{254}	Ethyl acetate-methanol-25% aqueous ammonia (60:30:10)	Densitometry at 280 nm	[22]
Azathioprine	Pharmaceuticals	TLC Silica gel 60 F_{254}	Methanol-toluene-25% ammonia (7:3:0.1)	Densitometry-285 nm	[23]
Irinotecan HCl	Pharmaceuticals	HPTLC Silica gel 60 F_{254}	Toluene-ethyl acetate-methanol-carbon tetrachloride (9.2:5:0.9:0.8)	Densitometry-317 nm	[24]
Tamoxifen citrate	Dissolution media	HPTLC Silica gel 60 F_{254}	_	Densitometry-258 nm	[25]
Bicalutamide	Bulk drug, liposomal formulation	HPTLC Silica gel 60 F_{254}	Toluene-ethyl acetate (4.5:5.5)	Densitometry-273 nm	[26]
Camptothecin	Liposomes	HPTLC Silica gel 60	Chloroform- methanol-triethylamine-water (30:35:34:8)	-10% $CuSO_4$ acidified with 85% H_3PO_4 -UV-254 and 366 nm	[27]
Doxorubicin	Liposomes	TLC Silica gel 60 F_{254}	Chloroform-methanol-NH_4OH (65:25:4) Chloroform-methanol-acetic acid- H_2O (25:15:4:2)	I_2 vapor	[28]
6-mercaptopurine, Daunorubicin	Liposomes	TLC Silica gel	Chloroform-methanol-water (70:25:5)	-	[29]
Aminoglutethimide acetyl and dansyl analogs	Standard compounds	TLC Silica gel 60 F_{254}	30% hydroxy trimethylpropylammo-nium-β-CD and methanol (50:50)	UV-254 nm	[38]

3. CHIRAL ANALYSIS OF ANTICANCER DRUGS

Enantiomer separations are one of the most important applications of TLC. Several reviews were published on this topic and a variety of chiral compounds as well as chiral anticancer drugs were presented [33-35].

Lepri et al. enantioseparated aminoglutethimide on triacetyl cellulose using the mobile phases containing ethanol or 2-propanol [36]. In another study, the use of derivatizing reagents to produce diastereoisomers which can be resolved by conventional phases was described, and cyclophosphamide was chromatographed using a derivatization reagent, (-)-1-phenethyl alcohol [37].

Racemic aminoglutethimide and its dansyl and acetyl analogs were separated and determined by TLC. Experimental procedures of this method are given in Table 1. Mobile phase composition was found to be very important for enantiomeric resolution [38].

4. ANALYSIS OF ANTICANCER DRUGS USING TLC METHOD IN BIOLOGICAL FLUIDS

Several reviews were published for the bioanalysis of different anticancer agents as a part of a special issue. These articles present an overview of the separation techniques including TLC method [39-43].

Boddy and his group published several papers reporting the metabolism of ifosfamide and cyclophosphamide. In these studies, the main drugs and their metabolites were determined in plasma and urine samples [44-48]. Experimental procedures of these methods are given in Table 2.

A "P-postlabelling method" was used by Koskinen et al. for the analysis of DNA isolated from livers of rats receiving tamoxifen. The postlabelled DNA was analysed by TLC on polyethyleneimine plates followed by autoradiography [49].

Surface Enhanced Raman Spectroscopy (SERS), used for detecting molecules at very low concentrations, is a promising technique for biomedical sensing applications. The validity of coupling TLC to SERS has been demonstrated for the detection of a great variety of substances, from environmental aromatic pollutants, to antidiabetic drugs, totobacco-related biomarkers and to alkaloid dyes. Vicario et al. reported the coupling of SERS to TLC for the determination of anticancer agent irinotecan in presence of human serum albumin [50].

Bhusari et al. conjugated trastuzumab with a bifunctional chelator, cyclic diethylene triamine-pentaacetic anhydride (cDTPAA) for radiolabeling with Tc-99m. By this way, a radio-pharmaceutical was developed and radio TLC was used for the quality control of this preparation [51]. In another report, ^{99m}Tc-paclitaxel was synthesized and its radiochemical purity was validated by TLC scanner. In vitro stability of the ^{99m}Tc-paclitaxel complex was determined in phosphate buffer saline (pH 7.4) and in rat serum separately. The samples were analyzed by using ascending instant TLC [52]. Recently, Monteiro et al. developed ^{99m}Tc-labeled paclitaxel and used a TLC method to evaluate the radiochemical purity and in vitro stability of ^{99m}Tc-paclitaxel in saline and murine plasma [53].

Apart from these methods; pyrimidine antimetabolites, cytarabine, ftorafur, 6-azauridine, 5-fluorouracil, trifluorothymidine, and two metabolites uracil arabinoside (metabolite of cytarabine) and uracil (metabolite of ftorafur), extracted from plasma, were separated by TLC on silica gel. The substances were visualized by UV irradiation [54].

In another study, the pharmacokinetics of carmustine, 4-hydroperoxycyclophosphamide, and paclitaxel was investigated in the monkey brain. Drug concentrations were determined in the brain, blood, and cerebrospinal fluid by quantitative autoradiography, TLC, and scintillation counting [55].

5. ANALYSIS OF ANTICANCER DRUGS USING TLC METHOD IN PLANT MATERIALS

Taxol (paclitaxel), is a diterpene isolated from *Taxus brevifolia*. In 2003, a book titled "Taxus: The Genus Taxus" was published including their production, biosynthesis and the analytical methods for their analysis [56]. Moreover, TLC of taxanes was presented in a chapter of book titled "Thin Layer Chromatography in Phytochemistry" [57].

Various TLC methods were developed for different purposes including isolation, purification, and/or quantitation of taxanes [58-70]. Experimental procedures of these methods are given in Table 3.

Wang et al. determined paclitaxel using TLC method with experimental design. Also, the mixture of dichloromethane and ethanol (1:1) was found to be the best extraction solvent [71].

Apart from taxanes, the extracts of *N. foetida* and *P. hexandrum* were analyzed by TLC for the content of camptothecin and podophyllotoxin [72-75].

Table 2. TLC Methods of Anticancer Drugs in Biological Fluids.

Compounds	Sample	Adsorbent	Solvent System	Detection	Ref.
Cyclophosphamide and its metabolites	Plasma and urine samples	HPTLC Silica gel	Butanol-water (20:3) or Chloroform-ethanol-glacial acetic acid (20:5:0.1) and Dichloromethane-methanol-glacial acetic acid (18:12:0.1)	15% NBP in acetone and acetate buffer (pH 4; 8:2)	[44]
Cyclophosphamide and its metabolites	Plasma, urine samples	HPTLC Silica gel	Butanol-water (20:3)	5% NBP in acetone and acetate buffer (pH 4; 8:2)	[45]
Ifosfamide and its metabolites	Urine, plasma and cerebrospi-nal fluid samples	HPTLC Silica gel 60	Dichloromethane-methanol-glacial acetic acid (90:8:1) and Chloroform- methanol-glacial acetic acid (90:60:1)	5% NBP in acetone-0.2 M acetate buffer (pH 4.6; 8:2)	[46]
Ifosfamide and its metabolites	Plasma, urine samples	TLC Silica gel	Dichloromethane-dimethyl formamide-glacial acetic acid (90:8:1) and Chloroform-methanol-glacial acetic acid (90:60:1)	5% NBP in acetone-0.2 M acetate buffer, (pH 4.6; 8:2)	[47, 48]
Irinotecan	Albumin solution	TLC Silica gel 60	Chloroform-methanol (84:16)	UV-254 nm	[50]
Paclitaxel (Radiolabeled)	Rat serum	TLC Silica gel	Acetone	-	[52]
Paclitaxel (Radiolabeled)	Murine plasma	TLC Silica gel	Acetone	-	[53]
Cytarabine, ftorafur, 6-azauridine, 5-fluorouracil	Human plasma	TLC Silica gel 60 F_{254}	Butanol-isopropyl alcohol-water (7:1:2)	UV-254 nm	[54]
Carmustine, 4-hydroperoxy cyclophosphamide (4-HC) and paclitaxel	Monkey brain, blood and CSF	TLC Silica gel	Chloroform (carmustine), Acetone-chloroform (1:1) (4-HC), Acetone-chloroform (1:3) (paclitaxel)	Scintillation counting	[55]

Endophytic fungi are symbiotically live on plants and they can synthesize the same bioactive compounds as their host plant themselves. Thus, they are investigated for the production of valuable anticancer agents. Kumar et al. determined vinblastine and vincristine from the endophytic fungus *Fusarium oxysporum* isolated from *C. roseus* found in India. The purification processes were performed with preparative TLC and HPLC [76]. Recently, another endophytic fungal compound, camptothecin was investigated. Endophytic fungi were isolated from *C. acuminata* and camptothecin from strain S-019 was characterized by different methods including TLC [77].

The use of biotransformation for the production of anticancer compounds is very useful. For instance, vincristine is more valuable and less abundant anticancer drug compared to vinblastine. Kumar and Ahmad described the production of vincristine using vinblastine by an endophytic fungus *Fusarium oxysporum* isolated from the plant *Catharanthus roseus* and analysed the transformed compounds using TLC [78]. In another study, the intermediates and the products of this process were detected by the combination of TLC and HPLC [79].

Apart from these well known plant materials with anticancer activities; Khabiya et al. investigated three lignans (phyllanthin, hypophyllanthin, and niranthin) of *Phyllanthus amarus* which possesses a wide variety of pharmacological activities including anticancer activity. A simple HPTLC method was developed for the simultaneous quantification of these lignans from the whole plant of *P. amarus* on TLC silica gel 60 F254 layers [80].

6. DETERMINATION OF LIPOPHILICITY

The determination of lipophilicity of drugs is extremely important because it defines many properties of a drug including solubility, transcellular permeability, distribution, target protein binding and plasma protein binding [6]. TLC is widely used for lipophilicity measurement and many examples of this application are given in this review.

Table 3. TLC Methods of Anticancer Drugs in Plant Materials

Compounds	Sample	Adsorbent	Solvent System	Detection	Ref.
Taxanes	*T. baccata*-needles and stems	TLC Si60 F_{254s} RP18W F_{254s} and HPTLC NH_2 F_{254s}	Heptane-ethyl acetate (5:5), Methanol-water (8:2), Chloroform-acetone (15:5)	UV-254 nm, 366 nm	[62]
Taxol	Endophytic fungi (strain TF5)	TLC Silica gel	Chloroform-methanol (7:1), Chloroform-acetonitrile (7:3)	H_3PO_4-ethanol (2:8), H_2SO_4-methanol (1:1) and others	[63]
Taxol and 10-DABIII	*Glicladium* sp. isolated from *T. baccata*	TLC Silica gel G	Chloroform-methanol (7:3), Chloroform-acetonitrile (7:3) (Prep. TLC)	Anisaldehyde-sulfuric acid or vanillin- sulfuric acid	[64]
Taxol	*T. baccata*-roots	TLC Silica gel	Water saturated ethyl acetate (I) Chloroform-methanol (95:5) (II) Ethyl acetate-methanol-water (100:5:1) (III)	UV-230 nm	[65]
Taxanes: 10-deacetyl-baccatin III, baccatin III, cephalomannine, paclitaxel	*Taxus* species-twigs, crude extracts or CC isolated fractions	TLC Silica gel 60 F_{254}	Benzene-chloroform - acetone-methanol (20:92.5:15:7.5), (8:37:6:3) Dichloromethane-dioxane-acetone-methanol (84:10:5:1)	UV-254 nm, Densitometry at 230 nm UV-366, 254, 230 nm	[66] [58] [59] [60] [61]
Taxanes	*Taxus*-twigs and needles	TLC Silica gel 60 HF_{254}	Heptane-dichloromethane-ethyl acetate (50:40:5)	UV-254 nm	[67]
Taxanes	*Taxus*-needles	TLC Silica 60	Heptane-methanol-chloroform (60:5:95, 70:5:95)	Densitometry at 243 nm	[68]
Taxanes	*T. chinensis*, *T. baccata*-cell cult.	TLC Silica gel GF_{254}	Chloroform-acetonitrile (4:1)	UV-254 nm, or vanillin-sulfuric acid	[69]
Taxol	*Taxus wallichiana*	TLC Silica gel GF_{254}	Chloroform- acetonitrile (7:3)	UV-254 nm, and vanillin-sulfuric acid	[70]
Paclitaxel	Taxus cuspidata	TLC Silica gel	Dichloromethane-ethanol (9:1)	UV-228 nm (Vanillin, sulfuric acid and ethanol)	[71]
Camptothecin	*N. foetida*-stem	TLC Silica gel 60 F_{254}	Toluene-acetonitrile-glacial acetic acid (6.5:3.5:0.1)	Densitometry at 370 nm	[72]
Podophyllotoxin	*P. hexand rum* Royle-tissue culture	TLC RP18 F_{254}	Acetonitrile-water (50:50)	Densitometry at 217 nm	[73]
Camptothecin	Callus and *N. foetida*-various parts	TLC Silica gel 60 F_{254}	Chloroform-ethyl acetate-methanol (4:5:0.5)	UV-360 nm	[74]
Podophyllotoxin	*P. hexand rum*-callus and roots	TLC Silica gel GF_{254}	Acetonitrile-water (4:6)	UV-210 nm	[75]
Vincristine and vinblastine	Endophytic fungi (from *C. roseus*)	TLC Silica gel-G	Chloroform-methanol (8:2)	Ceric ammonium sulphate	[76]
Camptothecin	Endophytic fungi (strain S-019)	TLC Silica gel	Chloroform-methanol (9:1)	UV detection	[77]
Vincristine	Endophytic fungi (from *C. roseus*)	TLC Silica gel-G	Chloroform- methanol (8:2)	Ceric ammonium sulphate	[78]
Vinorelbine	Standard material	TLC Silica gel GF_{254}	Petroleum ether-chloroform-acetone-diethyl amine (23.5:12:2:2.5)	UV-254 nm	[79]

Recently, a review was published presenting the principles of quantitative structure-retention relationships (QSRR) used for lipophilicity prediction from retention data. Moreover, the use of these data in quantitative structure-activity relationship (QSAR) studies was discussed [81]. In another recent review, the unconventional TLC systems in lipophilicity determination were discussed. These systems include: (1) the use of medium-polar stationary phases: CN, NH_2, and DIOL instead of RP plates, together with water-based mobile phase; (2) the use of silica gel in a typical normal-phase manner and treating extrapolated retention

indices as the "reversed lipophilicity"; (3) the use of oil impregnated silica gel in the reversed-phase manner; and (4) the use of salting-out mobile phases. It was reported that the chromatographic indices obtained in these systems are numerously reported as well correlated with lipophilicity and they are an interesting alternative to classical RP systems approaches [82].

The lipophilicity of antineoplastic propargylthioquinoline derivatives was investigated using chromatographic and computational methods. They were chromatographed on C_{18} RPTLC stationary phase using acetonitrile-water mixture as mobile phase [83]. Perisic-Janjic et al. evaluated the lipophilicity of some dehydroepiandrosterone derivatives by HPTLC [84]. In another study, the same group applied normal-phase TLC retention data in QSAR studies and determined the structure of dehydroepiandrosterone derivatives [85].

The lipophilicity of 6-mercaptopurine and its derivatives (azathioprine, methylazathioprine and 6-methylmercaptopurine) was determined on HPTLC RP-18, F254s plates developed with the mixture of acetonitrile and water, with acetonitrile concentration ranging from 50 to 80%. Lipophilicity was established from linear relationships between solute RM values [RM = log (1-Rf / Rf)] and acetonitrile concentration [86]. The lipophilicity of 2,6-disubstituted 7-methylpurines and 6-mercaptopurine was determined by RPTLC on precoated RP-18F254 plates with mixtures of acetone and buffer (sodium acetate-veronal, pH 7.0) as mobile phases. RM values of all the compounds decreased linearly with increasing concentration of acetone in the mobile phase. Experimental lipophilicity (log *P*TLC) was determined by use of a calibration plot obtained for five standards. The partition coefficient Clog *P* was calculated for all the compounds by use of the software CS Chem3D and high correlation was achieved between experimental log *P*TLC and theoretical Clog *P* values [87]. RPTLC was also used for the determination of lipophilicity parameters of azathioprine and nineteen of its derivatives. Experimental values (RM and log *P*TLC) were compared with theoretical values (Clog *P*) obtained using 9 computational methods. Separation was carried on silica gel RP-18 F254S plates with acetone-TRIS buffer pH 7.4 mixtures containing acetone in the range of 40–80% (v/v) in 5% increments as mobile phases [88].

7. CONCLUSION

It has been shown that TLC is widely used in industrial and clinical laboratories for the analysis of anticancer drugs because it is a simple and versatile technique with low cost of analysis, minimal sample clean-up and high sample loading capacity. In TLC, multiple standards and samples can be chromatographed on adjacent lanes of a single plate (high throughput) with the ability to use a variety of detection and quantification methods on each chromatogram. Also, with the introduction of HPTLC plates; resolution and in situ quantification have been improved with shorter analysis time and higher detection sensitivity. Among the most active research areas of TLC that is growing quickly are TLC-densitometry, retention-lipophilicity studies, the preparation of nanostructure for ultrathin layers (UTLC), use of biological detection methods and TLC coupled with MS. In this review, an extensive survey of the literature has been conducted and many TLC methods of important anticancer drugs in pharmaceuticals, biological fluids and plant materials; chiral analysis, retention-lipophilicity studies and radiochemical purity of labeled anticancer drugs have been presented.

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