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Review

Analytical approaches for traditional Chinese medicines exhibiting antineoplastic activity

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Abstract

Traditional Chinese medicines have attracted great interest in recent researchers as alternative antineoplastic therapies. This review focuses on analytical approaches to various aspects of the antineoplastic ingredients of traditional Chinese medicines. Emphasis will be put on the processes of biological sample extraction, separation, clean-up steps and the detection. The problems of the extraction solvent selection and different types of column chromatography are also discussed. The instruments considered are gas chromatography, capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC) connected with various detectors (ultraviolet, fluorescence, electrochemistry, mass, etc.). In addition, determinations of antineoplastic herbal ingredients, including camptothecin, taxol (paclitaxel), vinblastine, vincristine, podophyllotoxin, colchicine, and their related compounds, such as irinotecan, SN-38, topotecan, 9-aminocamptothecin, docetaxel (taxotere) and etoposide, are briefly summarized. These drugs are structurally based on the herbal ingredients, and some of them are in trials for clinical use. Evaluation of potential antineoplastic herbal ingredients, such as harringtonine, berberine, emodin, genistein, berbamine, daphnoretin, and irisquinone, are currently investigated in laboratories. Other folk medicines are excluded from this paper because their antineoplastic ingredients are unknown. © 2001 Elsevier Science B.V. All rights reserved.

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Contents

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1. Introduction

Herbal medicines were one of the major resources for health-care in early eras. Some traditional herbal medicines have been used for a long period of time. The treatments of various diseases were recorded in the oldest Chinese herbal encyclopedia, Shen-Nong-Pen-Tsao in 1596. Currently, herbal medicines are gaining more attention from modem pharmaceutical institutes, as scientists become aware that herbal medicine is an almost infinite resource for drug development. There are approximate 250 000 species of plants, up to 30 million species of insects, and millions of microorganisms [1]. Each species of plant produces a number of secondary metabolites. Although only 6% of the species have been studied for biological activity, about 4000 new ingredients are reported every year [2,3].

Based on daily practice, traditional Chinese medicine has accumulated several thousand years of practical experience. These medicinal plants usually contain complex constituents, and possible one or some of the ingredients have potent anticancer properties. Therefore, efficient methods are required for separation and identification these anticancer ingredients from other components of plants, such as protein, amino acid, sugars, resin, alkaloids, flavones, etc. Analysis of herbal medicine is an important technique, which defines and investigates many applications in biochemical, pharmaceutical and clinical research. Analytical strategies of separation and identification of herbal medicines from biological fluids include thin-layer chromatography (TLC), gas chromatography (GC), gas chromatography–mass spectroscopy (GC–MS), high-performance liquid chromatography (HPLC), liquid chromatography–mass spectroscopy (LC–MS), capillary electrophoresis (CE), capillary electrophoresis coupled to laser-induced fluorescence (CE–LIF).

Therapeutic effects of antineoplastic Chinese medicine have been confirmed by contemporary scientific research using chemical, pharmacological, molecular biological, and clinical studies. This review paper summarizes the analytical methods of pretreatment, separation, and detection of traditional antineoplastic herbal ingredients from biological fluids.

2. Pretreatment and reversed-phase HPLC determination of antineoplastic Chinese medicine

The sensitivity, precision and specificity of HPLC make it suitable for the determination of antineoplastic agents in the pre-clinical research or routine hospital use. HPLC methods with various strategies, such as difference type of stationary phase, various

composition of mobile phase and sample preparation, have been reported for the determination of antineoplastic drugs in biological fluids. Recently, HPLC coupled to mass spectrometry has been developed for the measurement of antineoplastic drugs. This methodology is very sensitive in determining lower levels of a drug in biological fluids; however, it requires several further steps of procedures, such as nebulization and vaporization of liquid, ionization of sample, removal of the excess solvent, and transfer of the ionic molecule into the mass analyzer. The instrument is also very expensive and its operation requires considerable experience not readily available in most research laboratories. Therefore, it is excellent as a reference method, but may not be suitable for large population screening. The development of a liquid chromatographic method for the analysis of antineoplastic drug in biological fluids may be divided into the following steps.

2.1. Biological sample preparation

Biological fluids are complex mixtures, composed of proteins, peptides, lipids, amino acids, electrolytes and small molecules. Beyond the deleterious effects that these compounds may cause on the pump, the injector and the column, their presence will frequently interfere with the separation of the antineoplastic drugs. Consequently, selection of the sample preparation is always required before injection onto the HPLC system. For the quantitation of antineoplastic drugs in HPLC, sample pretreatment, including protein precipitation, solid-phase extraction on cartridges, and dilution of sample in an appropriate solvent or buffer have been used. The choice of pretreatment strategies and sample clean-up procedures will affect the efficiency and the selectivity of the chromatographic technique.

2.2. Protein precipitation

Proteins, which may cause chromatographic column obstruction, are the principle components of biological matrices. Therefore, protein removal is an essential procedure in the sample preparation process for HPLC. Protein precipitation procedures are popular and easy to perform prior to injection into HPLC or CE. Several methods for deproteinization of physiological samples have been described in the literature [4–6]. A variety of reagents (acids, salts and organic solvents) can be added to biological fluids to precipitate protein [7]. Most proteins and fibrin should be removed from plasma samples to prevent plugging of the column filter and packing materials. Protein precipitation also results in the release of the protein-bound portion of drugs. There is equilibrium between protein-free drugs and protein-bound drugs in blood, plasma or other biological samples. The sum of the bound and the free drug concentration is the total drug concentration. Therefore, the total drug concentration is measured by means of protein precipitation.

Analytical problems associated with precipitation procedures are sample dilution, incomplete protein precipitation, drug co-precipitation and catalyzed degradation of labile drugs. Trichloroacetic acid, perchloric acid, methanol, ethanol and acetonitrile are frequently used for deproteinization of biological samples. Recently a simple protein precipitation with acetonitrile was used for the determination of the total forms of irinotecan and its active metabolite SN-38 in human serum, using liquid chromatography–electrospray mass spectrometry (LC–ES-MS) method [8]. The sample treatment involves a protein precipitation of 1:2 (v/v) volume ratio of samples with acetonitrile.

Precipitation procedures with strong acids might threaten the structural integrity of the antineoplastic drugs. de Bruijn et al. [9] demonstrated a simple protein precipitation with aqueous perchloric acid–methanol (1:1, v/v), which results in the conversion of the carboxylate to the lactone forms of the antitumor agent irinotecan (CPT-11) and its active metabolite SN-38, in human plasma.

In general, several parameters must be considered before choosing the types of protein precipitation. First of all, deproteinization by trichloroacetic acid or other acids results in only a small degree of dilution. Secondly, the volume of additive needed to precipitate a given proportion of proteins causing sample dilution. In contrast, four or five volumes of a concentrated salt solution (e.g., ammonium sulfate) are required to adequately deproteinize one volume of plasma. One should be able to decide an appropriate dilution according to analytical needs. Finally, salt or acid additives leave analytes in a high-con-

ductivity matrix but the supernatant after precipitation with organic solvent has a low conductivity. Conductivity is an essential element for electrochemical detection (ED) and electrophoresis, which should be taken into account by an analytical detection system.

The injection of a high percentage of organic solution may transiently disturb the resolution of the column, causing a broadening of bands and short analysis time of antineoplastic drugs. Under these conditions, peak areas remained unchanged. The broadening was more marked with ethanol than with methanol.

Another disadvantage of the deproteinization methods stems from the dilution, which leads to a decrease in the sensitivity. When using an organic solvent, one way to overcome this problem is to back-extract the excess solvent. Then, after the deproteinization of plasma or serum using an equal volume of acetonitrile, the additive acetonitrile is removed from the supernatant by methylenechloride. Then, the antineoplastic drug is concentrated in the supernatant.

2.3. Liquid–liquid phase extraction

In liquid–liquid phase extraction, an immiscible organic phase is added to the biological fluids. These mixture are then shaken together, resulting in the more hydrophobic sample components being extracted into the organic phase. The inorganic salts present at high concentrations in most biological fluids are absent from the extract. This strategy is very useful from the viewpoint of mass spectrometry and CE [10]. The application of liquid–liquid phase extraction in the simultaneous determination of lactone and carboxylate forms of the novel product anticancer agent 10-hydroxycamptothecin with recoveries of greater than 90% in plasma and urine was reported [11].

2.4. Liquid–solid (solid phase) extraction

Differences in affinity of various molecules to active sites located on the surface of sorbent materials resulting in separation of mixtures of different molecules are the basic theory of solid-phase extraction. The first step of this clean-up procedure is adsorbing drugs into either an ion-exchange resin, if they were charged, or bonded reversed-phase packing materials. With respect to the type of the bonded phases, octadecyl-bonded silica has become the most popular phase. Others, such as C_2, C_4, C_8, phenyl, cyanopropyl, diphenyl and diol were also published for various extractions. In the quantitation of total and lactone 20(*S*)-camptothecin, the adsorbing interaction with packing material in the pre-column was stronger than the interaction between antineoplastic drugs and protein, the extraction efficiencies were about 100% [12]. In the column-switching technique, a fraction of the effluent from a primary column was selectively transferred to an analytical column. For the simultaneous determination of the camptothecin (CPT) derivative, irinotecan hydrochloride (CPT-11) and its metabolites, 7-ethyl-10-hydroxycamptothecin (SN-38) and SN-38 glucuronide (SN-38G) in rat plasma, automation of the solid-phase extraction of drugs has been developed [13].

Liquids used in liquid–liquid phase extraction and solid-phase extraction can be evaporated away, and the residue then re-dissolved in a small volume of liquid. Most of the solid-phase extraction procedures described for the extraction of anticancer drugs are off-line [12,14,15]. However, fully automated on-line solid-phase extraction system has been developed for the simultaneous determination of the camptothecin (CPT) derivative, irinotecan hydrochloride (CPT-11) and its metabolites, 7-ethyl-10-hydroxycamptothecin (SN-38) and SN-38 glucuronide (SN-38G) in rat plasma. Compared with conventional methods, the automatic method has several advantages, i.e., rapid and simple sample preparation with a fully automated on-line solid-phase extraction system, as well as being time-saving due to the simultaneous determination of irinotecan and its metabolites [13].

2.5. Microdialysis for protein-free drug sampling

Microdialysis sampling from the biological fluids is based on the diffusion of analytes from interstitial tissue into the semipermeable membrane of a microdialysis probe [16–18]. These membranes have a molecular mass cut-off typically from 6 to 42 kDa. When small molecules pass through the microdialysis membrane, large molecules are excluded. Sampling and determination of the protein-unbound

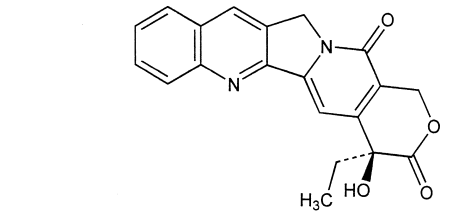
fraction of a drug in a biological system is of utmost pharmacological importance. In the past two decades, microdialysis sampling has become an important technique allowing the in vivo sampling from extracellular fluids. Analytical consideration is another major concern since microdialysis is a continuous process. In general, microdialysis offers advantages in terms of maintaining equilibrium and experimental versatility in vivo for time-dependent sampling, continuous sampling avoiding enzymatic degradation of the sample, and sampling in awake, freely moving animals. Microdialysis samples can then be collected for subsequent analysis or assayed online by HPLC or other suitable techniques. Recently, microdialysis and allied techniques have been used in the measurement and pharmacokinetic studies in rat blood, brain and bile [19,20].

3. Overview of antineoplastic herbal medicines

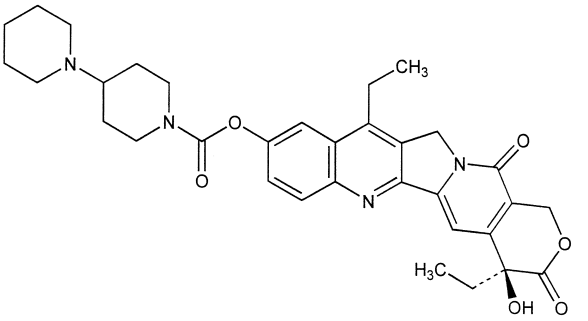
3.1. Analysis of camptothecin and its related compounds

Camptothecin (Fig. 1), a plant alkaloid, was first isolated from *Camptotheca acuminata* by Wall et al. [21–23] and the compound has shown significant antineoplastic activity in various experimental tumor models. However, the major dose-limiting toxicity led to the abandonment of further clinical testing from 1970 to 1980 [24]. Subsequently this cytotoxic effect of camptothecin has been shown to be targeted to the topoisomerase I [25], which is important for cancer chemotherapy [26]. In the late 1980s the camptothecin analogues attracted renewed interest because of the identification of the enzyme DNA topoisomerase I as a cellular target of this drug class. Based on the chemical structure of camptothecin, several anticancer drugs have been developed, including irinotecan (CPT-11) which its metabolite 7-ethyl-10-hydroxycamptothecin (SN-38), topotecan, 9-aminocamptothecin (NSC 603071) and 9-nitrocamptothecin, etc. [27,28]. In 1989, it was found that camptothecin and some of its semisynthetic derivatives possessed extraordinary anticancer activity against human cancer xenografts in nude mice [29].

Camptothecin has a pentacyclic indole moiety, which appears to be a principal requirement for its activity. However, this lactone ring is labile in aqueous solutions and undergoes reversible hydrolysis to the carboxylate form at pH values greater than the pK_a. Camptothecin solution is primarily in the lactone form at pH levels below 5 and converts predominantly to the ionized carboxylate form at pH values greater than 8. The human plasma pH value is around 7.4; therefore, a fractional amount of camptothecin should be converted to the inactive carboxylate form. At pH 7.4, human serum albumin preferentially binds the carboxylate form with a 150-fold higher affinity than the lactone form [30]. Although the carboxylate form of camptothecin shows preferential binding with the plasma albumin leading to stabilization of this form, the camptoth-



Camptothecin



Irinotecan

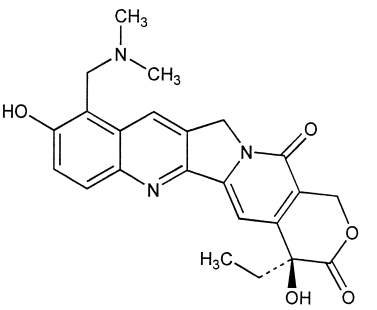


Fig. 1. Chemical structures of camptothecin, irinotecan and topotecan.

ecin lactone partitions into the lipid bilayers of erythrocytes, with the drug locating in a hydrophobic environment protected from hydrolysis [30]. Therefore, it is of particular interest to determine both lactone form and carboxylate form of camptothecin in clinical applications. In general, the separation and detection of camptothecin and its derivatives were performed by reversed-phase HPLC coupled to fluorescence detection. The described HPLC methods for camptothecin and its derivatives are summarized in Table 1.

The separations of the lactone and carboxylate forms of camptothecin or its derivatives using HPLC have often required mobile phases containing the following components. An ion-pairing reagent was used to provide adequate retention of the carboxylate form of the drug and a proper buffer was use to control the ionic strength and pH of the mobile phase. An appropriate organic solvent, such as acetonitrile to control the retention of the lactone form and sodium dodecyl sulphate (SDS) was used to reduce peak tailing [31]. Loh and Ahmad [32] used a potassium phosphate buffer method, and Supko and Malspeis [33] used an ammonium acetate buffer–acetonitrile–SDS system as the mobile phase for the separation of lactone and carboxylate forms of camptothecin. Although the mobile phase selection is somewhat similar for the camptothecin determination. Later, ion-pairing agent such as tetrabutylammonium phosphate was added to the mobile phase for the separation of the lactone and carboxylate species of camptothecin [4,34]. Recently, a simple HPLC methodology for the simultaneous separation of the lactone and carboxylate forms of numerous camptothecin in less than 10 min has been developed which includes only triethylamine acetate (TEAA) buffer and acetonitrile in the mobile phase. The content of the mobile phase for that separation was 23:77 (v/v) for acetonitrile–TEAA buffer (1%), pH 5.5. In that application, triethylamine acted as the ion-pairing reagent, masking underivatized silanols and also serving as the major buffer component. By

Table 1
HPLC assay for camptothecin and its derivations

Compound	Specimen	Column	Detection	Sample pretreatment	Reference
Camptothecin	Mice, plasma, heart, liver, spleen, lung, brain, kidney	RP-C_{18}	Flu, Ex 360 nm, Em 430 nm	SPE	[44]
Camptothecin	Rat bile	Micro RP-C_{18}	Flu, Ex 360 nm, Em 440 nm	Microdialysis	[19]
Camptothecin	Rat blood, brain	RP-C_{18}	Flu, Ex 360 nm, Em 440 nm	Microdialysis	[20]
Camptothecin	Mice plasma, urine	RP-C_{18}	Flu, Ex 370 nm, Em 434 nm	LLE	[32]
Camptothecin	Rat plasma	RP-C_{18}	Flu, Ex 369 nm, Em 426 nm	Protein precipitation	[4]
Camptothecin	Human plasma	RP-C_{18}	Flu, Ex 360 nm, Em 440 nm	SPE	[12]
Irinotecan	Human serum	RP-C_{18}	LC–ES–MS	Protein precipitation	[8]
Irinotecan	Rat serum	RP-C_{18}	Flu, Ex 373 nm, Em 428 nm	SPE	[13]
Irinotecan	Human plasma	RP-C_{18}	Flu, Ex 362 nm, Em 425 nm	Protein precipitation	[5]
Irinotecan	Human plasma	RP-C_{18}	Flu, Ex 370 nm, Em 432 nm	SPE	[45]
Irinotecan	Human plasma, urine, feces	RP-C_{18}	Flu, Ex 228 nm, Em 450 nm	Protein precipitation	[6]
Irinotecan	Human plasma	RP-C_{18}	Flu, Ex 355 nm, Em 515 nm	Protein precipitation	[9]
Irinotecan	Human plasma, urine	RP-C_{18}	LC–APCI–MS	LLE	[46]
SN-38	Human plasma	RP-C_{18}	Flu, Ex 375 nm, Em 560 nm	Protein precipitation	[5]
SN-38	Human plasma	RP-C_{18}	Flu, Ex 380 nm, Em 532 nm	SPE	[45]
SN-38	Human plasma, urine, feces	RP-C_{18}	Flu, Ex 228 nm, Em 543 nm	Protein precipitation	[6]
Topotecan	Human plasma, urine, feces	RP-C_{18}	Flu, Ex 380 nm, Em 527 nm	LLE	[47]
Topotecan	Human plasma, urine	RP-C_{18}	Flu, Ex 381 nm, Em 525 nm	LLE	[48]
Topotecan	Rat brain tissue	RP-C_{18}	Flu, Ex 380 nm, Em 520 nm	Microdialysis	[49]
9-Aminocamptothecin	Human plasma	RP-C_{18}	Flu, Ex 370 nm, Em 450 nm	LLE	[50]
9-Aminocamptothecin	Human plasma	RP-C_{18}	Flu, Ex 370 nm, Em 450 nm	SPE	[51]

altering only the composition of TEAA buffer with respect to acetonitrile, method development time may be reduced markedly [31].

3.1.1. Camptothecin assay

Typical chromatograms of standard containing camptothecin are shown in Fig. 2. Separation of camptothecin from endogenous chemicals in blood dialysate was achieved in an optimal mobile phase containing 20% methanol, 30% acetonitrile and 50% 1 m*M* octanesulfonic acid (pH 4.8, adjusted with orthophosphoric acid). The liquid chromatographic system consisted of a chromatographic pump (Waters 510, Bedford, MA, USA), an injector (Rheodyne 7125, Cotati, CA, USA) equipped with a 20-μl sample loop, and a fluorescence detector (Jasco FP-920, Tokyo, Japan). Dialysates were separated using a reversed-phase C_{18} column (Merck, 250×4.6 mm I.D.; particle size 5 μm, Darmstadt, Germany) maintained at an ambient temperature to perform the ideal chromatographic phase. The optimal fluorescence response for camptothecin was observed at excitation and emission wavelengths of 360 and 440 nm, respectively. Output data from the detector was integrated using an EZChrom chromatographic data system (Scientific Software, San Ramon, CA, USA). The chromatographic method was modified from our previous reports [19,20].

Fig. 2A shows a standard injection of camptothecin (50 ng/ml), and Fig. 2B shows the chromatogram of a blank blood dialysate. None of the observed peaks interfered with the analysis of the compound. Fig. 2C shows the chromatogram of a rat blood dialysate sample containing camptothecin (94 ng/ml) collected 24 min after camptothecin administration (5 mg/kg, i.v.). None of the observed peaks interfered with the analyte in the chromatogram of brain sample.

Fig. 3A shows a standard injection of camptothecin (50 ng/ml), and Fig. 3B shows the chromatogram of a blank brain dialysate. Fig. 3C shows the chromatogram of a brain dialysate sample containing camptothecin (16 ng/ml) collected from the rat brain microdialysate 24 min after camptothecin administration (5 mg/kg, i.v.).

Fig. 4A shows a standard injection of camptothecin (100 ng/ml). Fig. 4B shows a chromatogram of a blank bile dialysate sample obtained from bile duct microdialysis before the drug administration. Fig. 4C shows the chromatogram of bile dialysate sample obtained containing camptothecin (58 ng/ml) col-

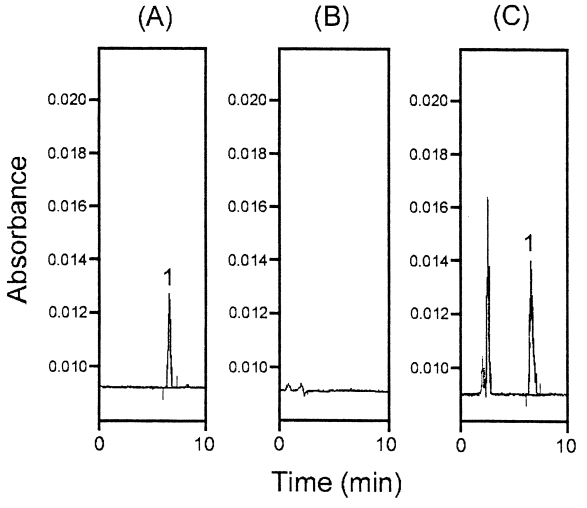


Fig. 2. (A) A standard injection of camptothecin (50 g/ml), and (B) the chromatogram of a blank blood dialysate. None of the observed peaks interfered with the analysis of the compound. (C) The chromatogram of a rat blood dialysate sample containing camptothecin (94 ng/ml) collected 24 min after camptothecin administration (5 mg/kg, i.v.).

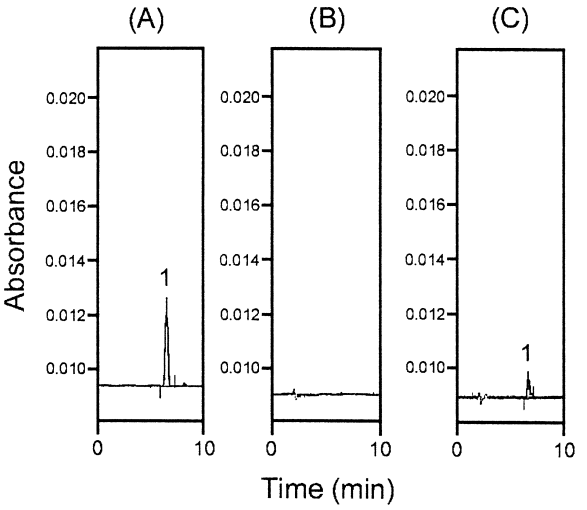


Fig. 3. (A) A standard injection of camptothecin (50 ng/ml), and (B) the chromatogram of a blank brain dialysate. (C) The chromatogram of a brain dialysate sample containing camptothecin (16 ng/ml) collected from the rat brain microdialysate 24 min after camptothecin administration (5 mg/kg, i.v.).

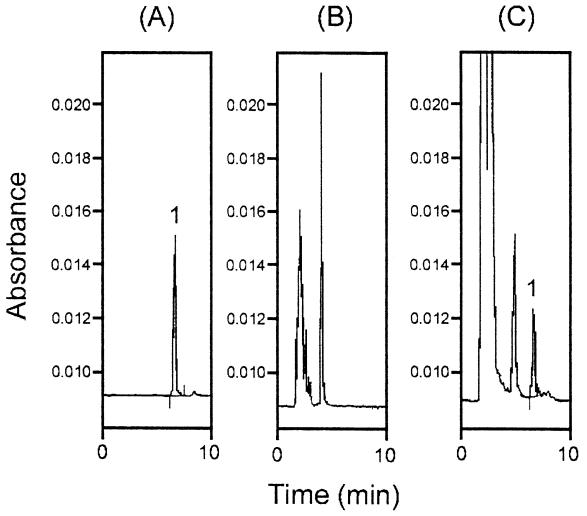


Fig. 4. (A) A standard injection of camptothecin (100 ng/ml). (B) A chromatogram of a blank bile dialysate sample obtained from bile duct microdialysis before the drug administration. (C) The chromatogram of bile dialysate sample obtained containing camptothecin (58 ng/ml) collected from the bile duct microdialysis 180 min after camptothecin administration (5 mg/kg, i.v.).

lected from the bile duct microdialysis 180 min after camptothecin administration (5 mg/kg, i.v.).

3.1.2. Irinotecan

Irinotecan (Fig. 1) and its more potent metabolite (SN-38; 7-ethyl-10-hydroxycamptothecin) are novel synthetic derivatives of the plant alkaloid camptothecin. These compounds interfere with mammalian DNA topoisomerase I and cancer cell death appears to result from DNA strand breaks caused by the formation of cleavable complexes. HPLC methods have been applied to assay irinotecan and its metabolites in various biological materials, and simultaneous determination of the carboxylate and lactone form of irinotecan or SN-38 is also possible [35–37]. It is essential to monitor the lactone and carboxylate forms of irinotecan since reports have suggested that the fraction of lactone to total (lactone plus carboxylate) is constant between patients [38,39]. Kurita and Kaneda[13] used a fully automated on-line solid-phase extraction system for the simultaneous determination of the camptothecin (CPT) derivative, irinotecan hydrochloride (CPT-11) and its metabolites, 7-ethyl-10-hydroxycamptothecin (SN-38) and SN-38 glucuronide (SN-38G) in rat plasma. Following this procedure, plasma samples were pretreated with 0.146 *M* H_3PO_4 to inactivate carboxylesterase and β-glucuronidase in rat plasma. This method has several advantages compared with conventional determination methods, such as automation of a complicated sample preparation, time-saving due to the simultaneous determination of irinotecan and its metabolites, and the small amount of plasma required for the determination [13].

3.1.3. Topotecan

Topotecan (Fig. 1), a semisynthetic water-soluble derivative of camptothecin, has been shown to be a potent inhibitor of DNA topoisomerase I in vitro and has demonstrated encouraging antitumor activity in a wide variety of tumors (ovarian cancer and lung cancer). It differs from its parent compound because it incorporates a stable basic side chain at the 9-position of the A-ring of 10-hydroxycamptothecin, which allows the drug to be formulated as a hydrochloric acid derivative, and which also makes it water soluble. The lactone ring is quite labile and topotecan undergoes a pH-dependent reversible hydrolysis of its lactone moiety, yielding a carboxylate form. Both lactone and carboxylate forms of topotecan have been reported for the quantitative analysis by HPLC in plasma and urine samples [40–42]. The analytical methodology reported involves a protein precipitation step with methanol as a sample pretreatment procedure. The instability of the drug in biological samples necessitates that the plasma fraction is obtained within 5 min after blood sampling by centrifugation, immediately followed by protein precipitation with cold methanol (−30°C) [40]. In addition, an acidified solution has been added for the determination of total topotecan levels (lactone plus lactone ring-opened form). The plasma samples were deproteinated with methanol and subsequently acidified with 2% (v/v) perchloric acid [43].

3.2. Analysis of taxol and its related compounds

Taxol (paclitaxel; Fig. 5) is a powerful antitumor drug which was first isolated from the bark of *Taxus brevifolia* Nutt [52]. However, the content of taxol in the tree was found to be 0.064 mg/g of dry powder, insufficient for its development as a drug [53].

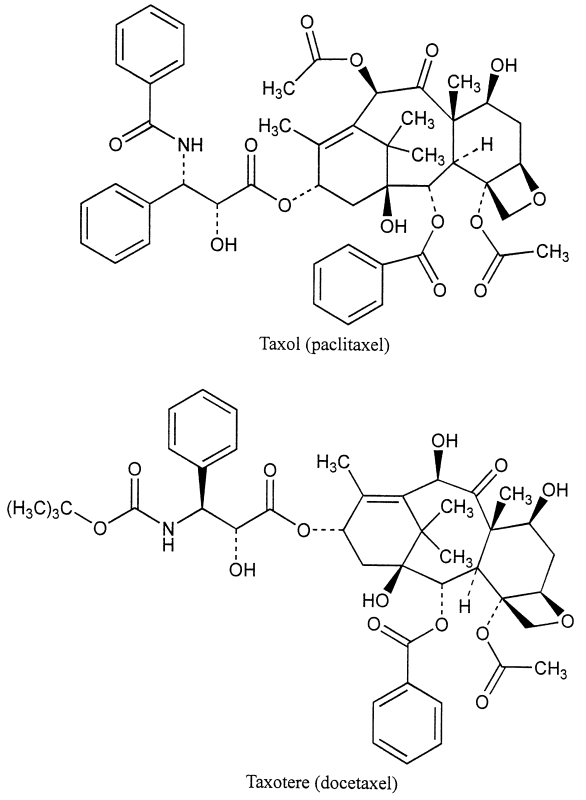


Fig. 5. Chemical structures of taxol and taxotere.

Alternatively, plant tissue cultures [54], and partial or complete chemical synthesis [55], are also being explored as other option.

A numbers of assay systems have been reported for the determination of taxol in the biological fluids using HPLC–UV, HPLC–MS, and immunoassay, with lower detection limits of 10, 0.2, and 0.3 ng/ml, respectively. Compared to the above three methods, HPLC–MS provides greater sensitivity and higher specificity than HPLC–UV. The immunoassay method gives high sensitivity but lacks the specificity of the HPLC method. Compensated with sensitivity and specificity, HPLC–UV is the most commonly performed method for the determination of taxol from biological fluids [56,57]. Recently, LC–MS–MS has been used to determine the concentration of taxol in various biofluids and is applicable to clinical pharmacokinetic studies [58]. This procedure is based on HPLC–ion spray–tandem mass spectrometry for sensitive, specific and accurate and quantification of the anticancer agent, taxol, in biological fluids [59]. The HPLC–APCI has been used for the determination of taxol, and the major human biliary metabolite in extracts of plasma and urine from patients with 50 pmol of separated compounds [60].

In order to exclude interference from the biofluids, a liquid–liquid phase extraction using *tert*.-butylmethyl ether was used for taxol sample manipulations in human plasma [57]. Solid-phase extraction alone [61], solid-phase extraction coupled to liquid–liquid phase extraction [62] or protein precipitation [63] have also been used for sample pretreatment. The described HPLC methods for taxol and taxotere assay are summarized in Table 2.

3.3. Analysis of vinblastine and vincristine

Vinblastine and vincristine (Fig. 6), antineoplastic vinca alkaloids, are isolated from the plant *Catharanthus roseus* [75,76]. Both alkaloids are commonly used in Western medicine for the treatment of breast, bladder, and lung cancers, lymphomas, leukemias and various neoplastic diseases. Current methods for the determination of vincas use HPLC with ultraviolet absorbance [77], fluorescence [78] or electrochemical detection [79]. Vendrig et al. [80] developed a fluorescence method with a solid-phase extraction on a cyanopropyl extraction column for the determination of plasma and urine. The influence of acetonitrile concentration and pH on the capacity factors of these drugs was studied in order to optimize the separation of the drugs from their endogenous components. The limit of determination for vinblastine is 0.5 ng/ml in plasma and urine, but the assay cannot measure low concentrations of vincristine [80]. A similar sensitivity has also been developed using electrochemical detection [81]. The linearity for vinblastine and vincristine assays was in the concentration ranges 1–100 ng/ml [81] and 1–1000 ng/ml [82].

The determinations of these compounds in biological fluids are of interest to various scientific fields. An on-line column switching using a C_{18} reversed-phase preconcentration column was developed for the determination of vincristine in human serum. After the on-line column extraction, both ultraviolet and electrochemical detection are possible, but the latter shows a cleaner chromatogram

Table 2
HPLC assay for taxol (paclitaxel) and its derivatives

Compound	Specimen	Column	Detection	Sample pretreatment	Reference
Taxol	Human plasma	RP-C_{18}	UV 227 nm	SPE	[57]
Taxol	Human plasma	RP-C_{18}	UV 230 nm	SPE	[64]
Taxol	Human whole blood	RP-C_{18}	LC–MS–MS	LLE	[59]
Taxol	Mouse plasma	RP-C_{18}	Diode array	LLE	[65]
Taxol	Rat serum	RP-C_{18}	UV 230 nm	LLE	[66]
Taxol	Human plasma, urine	Nucleosil-C_{18}	UV 227 nm	LLE	[67]
Taxol	Human plasma	RP-C_{18}	LC–MS–MS	SPE	[58]
Taxol	Human plasma	RP-C_{18}	UV 230 nm	LLE	[68]
Taxol	Rat bile	RP-C_{18}	LC–ISP–MS–MS	SPE	[69]
Taxol	Human plasma	RP-C_{18}	UV 227 nm	SPE	[70]
Taxol	Mouse plasma, urine, feces	APEX-C_8	UV 227 nm	SPE	[71]
Taxol	Human urine	RP-C_8	UV 227 nm	SPE	[72]
Taxol	Human plasma, cell culture, dog bladder	RP-C_{18}	UV 229 nm	SPE	[56]
Taxol	Mouse urine	μBondapak-C_{18}	Diode-array	LLE	[73]
Taxotere	Human plasma	RP-C_8	UV 227 nm	Column switching	[74]

with detection limit 0.3 ng/ml [83]. Vinblastine and 4-*O*-deacetylvinblastine have been determined in plasma and urine using ion-exchange normal-phase HPLC with fluorescence detection. The limit of detection was 1 ng/ml for both compounds using a 0.5-ml sample [84]. For the determination of vinblastine in various normal mouse tissues, such as lung, heart, liver, kidney and muscles, these tissues were treated with lyophilization. Following the process of pulverization and homogeneous mixing, the aliquot was suspended in 0.1 *M* hydrochloric acid. The detection limit of vinblastine in tissue was 10 ng/g [85].

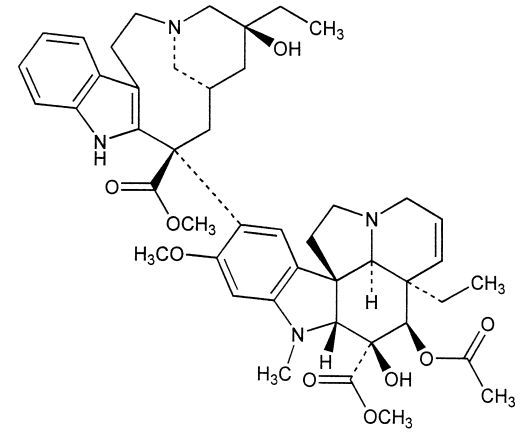
Vinorelbine is a semisynthetic vinca alkaloid agent that has been structurally modified on the catharanthine nucleus to impart increased lipophilicity. Catharanthine (Fig. 7) is an indole alkaloid found abundantly in the Madagascar periwinkle, *Catharanthus roseus* (formerly *Vinca rosea*), where it is the monomeric precursor of the dimeric anticancer drug vinblastine and vincristine [86]. Determination of vinorelbine in rabbit plasma has been developed using a liquid–liquid phase extraction coupled to a coulometric detection liquid chromatography. The limit of detection in plasma was 1 ng/ml. A linear response was observed for the plasma calibration graph in the ranges 2.5–50 and 50–1000 ng/ml [87].

Recently, liquid chromatography combined with atmospheric pressure chemical ionization mass spectrometry (APCI–MS) has provided a highly selective and sensitive method in a narrow concentration range 0.30–4.00 ng/ml. Determination was possible down to 0.51 ng/ml for vinblastine and 0.30 ng/ml for vincristine in human plasma using microbore separation column. The described HPLC methods for vinblastine, vincristine and vinorelbine assay are summarized in Table 3.

3.4. Analysis of podophyllotoxin-related derivatives

Etoposide (Fig. 8), a semi-synthetic derivative of podophyllotoxin (Fig. 8), is a highly active and widely used antineoplastic agent. Etoposide levels in the physiological fluids have been reported using HPLC coupled to various detectors such as UV [93], fluorescence [94–96], electrochemical detection [97–99], electrospray mass spectrometry [100] and ELISA [101]. For clinical application on cancer patients, the protein binding level of etoposide is lower than that of healthy subjects [102,103].

To assay etoposide from a biological matrix requires sample pretreatment. There are various methods for this sample preparation, such as liquid–liquid phase extraction with chloroform [96], di-



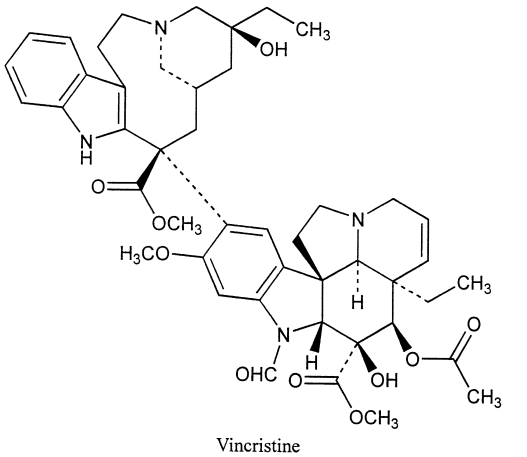


Fig. 6. Chemical structures of vinblastine and vincristine.

chloromethane [104], and ethylene dichloride [104]. Alternatively, a reversed-phase stationary phase of solid-phase extraction has been described for the clean-up of etoposide samples [105,106]. Since the protein-unbound form of drug concentration correlates with the concentration at the site of action, the free drug concentration is often considered as the best quantitation of the pharmacologically active drug. However, the protein-unbound etoposide concentration is often below the sensitivity of validated HPLC methods [94,107]. For these reasons, Robieux et al. [96] described a validated method for the measurement of protein unbound etoposide concentration in plasma using ultrafiltration and HPLC with fluorescence [96]. The described HPLC methods for podophyllotoxin and etoposide assay are summarized in Table 4.

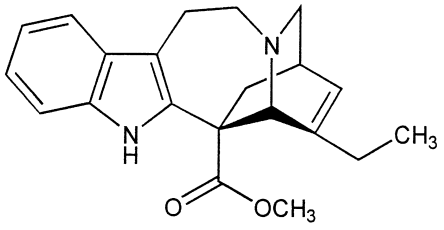


Fig. 7. Chemical structure of catharanthine.

3.5. Analysis of colchicine

Colchicine (Fig. 9) is a plant alkaloid, isolated from various species of *Colchicum* [112]. Its total chemical synthesis has been achieved; however, alcohol extraction of the alkaloid from the corm and seed of *Colchicum autumnale* L. currently remains the primary source of colchicine. The herb has been used for the relief of join pain since the 6th Century, and colchicine is currently indicated for the effective treatment of acute attacks of gout [113]. Colchicine has been proposed as an experimental antineoplastic agent [114].

Several methods have been reported for the determination of colchicine in biological fluids. Brues [115] uses a colorimetric method for colchicine assay. Subsequently, radioisotope dilution [116], radioimmunoassay [117], HPLC–UV [118], GC–MS [119] and HPLC–diode array [120] methods have been developed. Recently, an HPLC coupled with ion spray mass spectrometry (ISP–MS) has been developed for the determination of colchicine at ppb levels in human biofluids [121]. This method uses a single liquid–liquid phase extraction by dichloromethane at pH 8.0 for the clean-up of human blood, plasma or urine samples [121].

A sensitive radioimmunoassay (RIA) for plasma colchicine was developed with each antiserum with detection limit of 0.2 ng/ml. This RIA procedure appears suitable for plasma colchicine pharmacokinetics and monitoring investigations [122]. For the measurement of protein-unbound colchicine and its pharmacokinetics, a simultaneous brain and blood microdialysis system has been developed to study the passage of colchicine through the blood–brain barrier in the mouse [123] and freely moving

Table 3
HPLC assay for vinblastine and vincristine

Compound	Specimen	Column	Detection	Sample pretreatment	Reference
Vinblastine	Human plasma	RP-C_{18}	LC-APCI–MS	LLE	[88]
Vinblastine	Human plasma, urine, feces	Si	Flu, Ex 270 nm, Em 320 nm	LLE	[78]
Vinblastine	Mouse lung, heart, liver, kidney, muscles	LiChrosorb CN	UV 220 nm	LLE	[85]
Vinblastine	Human plasma, urine	Bond Elut CN	ED	SPE	[80]
Vinblastine	Human plasma	RP-C_8	UV 297 nm	SPE	[77]
Vinblastine	Human plasma, urine, brain, liver, kidney, intestine	RP-C_{18}	Flu, Ex 340 nm, Em 406 nm	LLE	[89]
Vinblastine	Human serum, urine	RP-C_{18}	ED	Column extraction	[83]
Vinblastine	Plasma	RP-C_{18}	Coulometric detection	LLE	[87]
Vinblastine	Tumor cells	Novapak C_{18}	Flu, Ex 280 nm, Em 360 nm	Protein precipitation	[90]
Vinblastine	Human plasma, urine	NP Silica	Flu, Ex 270 nm, Em 320 nm	LLE	[91]
Vinblastine	Human serum, urine	SGE CN	UV 268 nm	LLE	[92]

rats [124]. The concentration of colchicine dialysates was measured by the RIA method [125]. The described HPLC method for colchicine assay is summarized in Table 5.

3.6. Analysis of harringtonine and homoharringtonine

Harringtonine and homoharringtonine (Fig. 10) are two esters of the alkaloid cephalotaxine, isolated from the *Cephalotaxus* species [128,129]. Harringtonine has been reported to rapidly induce apoptosis in HL-60 cells in a wide scope/range of dosage with high chemotherapeutic efficiency to human chronic granulocytic and myelomonocytic leukemia [130]. Both compounds have also exhibited significant antileukemia activity and we widely used in clinics in China [131]. The extraction and determination of harringtonine and homoharringtonine from *Cephalotaxus* plant are done using counter-current chromatography [132], GC [133,134], GC–MS [133] and HPLC–UV [135,136]. The HPLC assay for harringtonine and its related compounds has been validated using reversed-phase C_{18} column in an alkali containing mobile phase [137]. To improve the tailing of homoharringtonine peak, triethylamine was added in the mobile phase and the pH was adjusted to 6.5 using 0.2% acetic acid [136].

In order to study the pharmacokinetics of harringtonine and homoharringtonine, a reliable analytical system is required. Jui and Roboz [138] developed an HPLC coupled to fluorescence detection for the measurement of harringtonine and homoharringtonine with limit of quantification of 30 and 10 ng/ml, respectively. However, this method is not significantly sensitive for further pharmacokinetic study. Roboz et al. [139] reported a mass spectrometric method for the determination of harringtonine and homoharringtonine with a detection limit of 10 ng/ml. Spencer et al. [133] reported a GC–MS method for the quantitation of harringtonine and homoharringtonine from the herbal extract. The sensitivity is often a major concern for the pharmacokinetic study. Later, a more sensitive HPLC coupled to electrochemical detection for the analysis of homoharringtonine in plasma with detection limit of 1 ng/ml. For further pharmacokinetic study, a single-step liquid–liquid phase partition extraction for homoharringtonine with dichloromethane was used for sample clean-up [140]. The described HPLC methods for harringtonine and homoharringtonine assay are summarized in Table 6.

3.7. Analysis of berberine

Berberine (Fig. 11) is a well-known isoquinoline alkaloid found in traditional Chinese herbal medicines such as *Coptidis chinensis* (Chinese name:

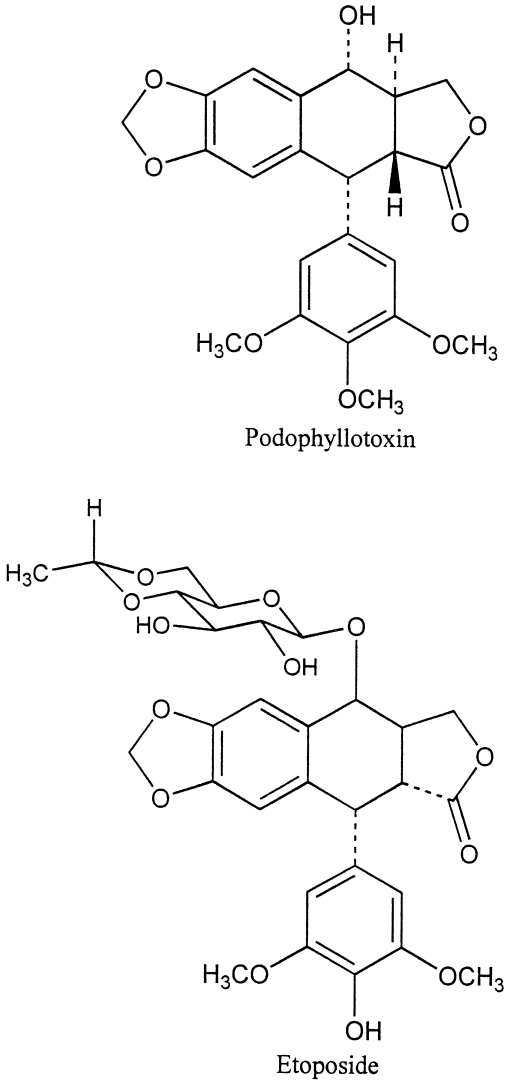


Fig. 8. Chemical structures of podophyllotoxin and etoposide.

huang-lien) which has been shown to induce apoptosis [141], and to induce differentiation of both human teratocarcinoma cells [142] and human esophageal cancer cell lines [143]. Analysis of berberine in medicinal plants using HPLC has also been reported [144,145]. Liebich et al. [146] used capillary zone electrophoresis (CZE) for the quantitative determination of the potential anticancer drugs berberine and isoguanosine in the extract of the traditional Chinese medicinal herbs [146]. These two compounds can be monitored at UV 254 nm using 100 m*M* sodium citrate running buffer (pH 2.7) and applied voltage 12 kV. Comparing the CZE and HPLC methods for berberine separation, CZE shows several advantages in higher separation performance over HPLC, such as rapidity of analysis, small sample volume, no requirement for organic solvent in the running buffer and low cost of reagents [146]. Recently, capillary electrophoresis–electrospray ion trap mass spectrometry has also been used for the determination of berberine in the Chinese herbal medicine [147]. In addition, the herbal ingredient identification and dubious adulteration have also been demonstrated by CE–ES–MS without extensive sample cleaning and preparation [147].

3.7.1. Microdialysis for berberine

Typical chromatograms of standards containing berberine are shown in Figs. 12–14. Separation of berberine from endogenous chemicals in blood, brain and bile dialysate were achieved in an optimal mobile phase containing 35% of acetonitrile and 65% of 20 m*M* monosodium phosphate (pH 5.0) and 0.1 m*M* 1-octanesulfonic acid. Liquid chromatographic-grade solvents and reagents were obtained from Merck (Darmstadt, Germany). Triply deionized water (Millipore, Bedford, MA, USA) was used for all preparations. The HPLC system consisted of a chromatographic pump (BAS PM-80, West Lafayette, IN, USA), an on-line injector (CMA 160, Stockholm, Sweden) equipped with a 10-μl sample loop and an ultraviolet detector (Dynamax, Walnut Creek, CA, USA). Berberine and dialysate were separated using a reversed-phase C_{18} microbore column (150× 1 mm I.D.; particle size 5 μm) maintained at ambient temperature. The optimal UV detection for berberine was set at a wavelength of 346 nm. Output data from the detector were integrated using an EZChrom chromatographic data system (Scientific Software, San Ramon, CA, USA).

Fig. 12A shows a standard injection of berberine (1 μg/ml). Fig. 12B shows a chromatogram of a blank blood dialysate. No peaks were observed that would interfere with the analysis of either compound. Fig. 12C shows a chromatogram of a blood dialysate sample containing berberine (3.48 μg/ml), which was obtained from blood microdialysis 20 min after berberine administration (20 mg/kg, i.v.).

Table 4
HPLC assay for podophyllotoxin and its derivative

Compound	Specimen	Column	Detection	Sample pretreatment	Reference
Podophyllotoxin	*Dysosma pleiantha*	Taxcil-C_{18}	UV 233 nm	Carbon dioxide extraction	[108]
Etoposide	Human plasma	RP-phenyl	ED	LLE	[99]
Etoposide	Human serum	Bondclone-C_{18}	Flu, Ex 230 nm, Em 330 nm	SPE	[106]
Etoposide	Human plasma	Hpersil RP-C_{18}	ED	Protein precipitation	[109]
Etoposide	Human plasma	Bondapak phenyl	Flu, Ex 288 nm, Em 328	Ultrafiltration	[96]
Etoposide	Human plasma, leukemic cells	Spherisorb phenyl	Flu, Ex 230 nm, Em 330	Ultrafiltration	[95]
Etoposide	Dog blood	RP-C_{18}	ED	LLE	[107]
Etoposide	Human plasma	Alltech RP-C_{18}	Flu, Ex 230 nm, Em 328	LLE	[110]
Etoposide	Human plasma	RP-C_{18}	UV 233 nm	LLE	[111]

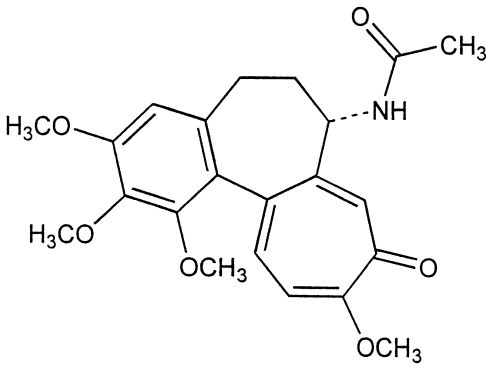


Fig. 9. Chemical structure of colchicine.

Fig. 13A shows a standard injection of berberine (0.1 μg/ml), and Fig. 13B shows the chromatogram of a blank brain dialysate. Fig. 13C shows the chromatogram of a brain dialysate sample containing berberine (0.058 μg/ml) collected from a rat brain microdialysate 20 min after berberine administration (20 mg/kg, i.v.).

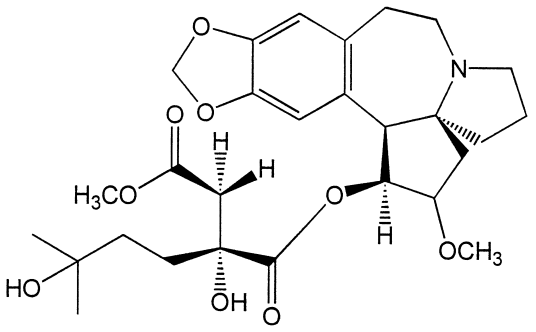
None of the observed peaks interfered with the analyte in the chromatogram of bile sample. Fig. 14A shows a standard injection of berberine (5 μg/ml). Fig. 14B shows a chromatogram of a blank bile dialysate sample obtained from bile duct microdialysis before the drug administration. Fig. 14C shows the chromatogram of bile dialysate sample obtained berberine (3.65 μg/ml) collected from the bile duct microdialysis 90 min after berberine administration (20 mg/kg, i.v.). The described HPLC method for berberine assay is summarized in Table 7.

3.8. Emodin

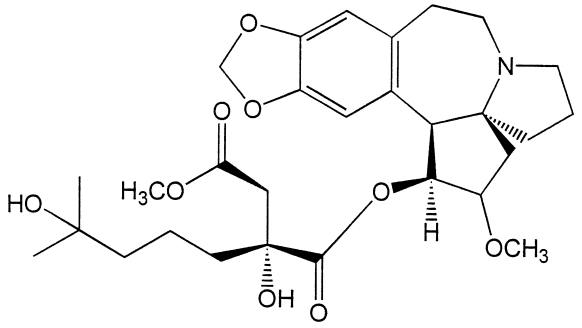
Emodin (1,3,8-trihydroxy-6-methylanthraquinone; Fig. 15) is one of the components which was isolated from Chinese herbal medicine *Rheum officinale* Baill (Chinese name: Da-Huang) and has specific in vitro and in vivo antineuroectodermal tumor activity [152]. This herb is commonly employed as a purgative crude herb in traditional herbal therapy [153].

Table 5
HPLC assay for colchicine

Compound	Specimen	Column	Detection	Sample pretreatment	Reference
Colchicine	Hippocampus		RIA	Microdialysis	[123]
Colchicine	Frontal cortex, blood		RIA	Microdialysis	[124]
Colchicine	Urine, serum	Microbore C_{18}	LC–ESP–MS	LLE	[126]
Colchicine	Serum	Microbore C_{18}	LC–ISP–MS	LLE	[121]
Colchicine	Serum, urine	RP-C_{18}	UV 254 nm	SPE	[127]



Harringtonine



Homoharringtonine

Fig. 10. Chemical structures of harringtonine and homoharringtonine.

Several methods have been reported for the determination of emodin, including capillary electrochromatography [154], liquid chromatography coupled with atmospheric pressure chemical ionization mass spectrometry [155], capillary electrophoresis [156,157] and HPLC–UV [158–161]. The described HPLC method for emodin assay in biological fluids is summarized in Table 8.

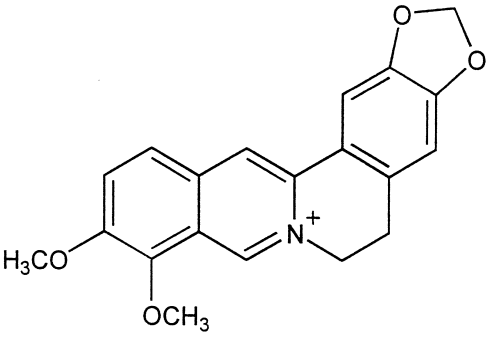


Fig. 11. Chemical structure of berberine.

3.9. Genistein

Genistein (4′,5,7-trihydroxyisoflavone; Fig. 16) is a potential isoflavone for the prevention and treatment of cancer [164,165]. Genistein has been found in soybean, which has led to the proposal that dietary consumption of soy products may prevent the development of breast cancer and delay progression of latent prostatic carcinoma [166,167]. Several chromatographic methods have been reported for the determination of genistein in various detectors such as UV [168,169], electrochemical detection [170], LC–MS [171,172] and GC–MS [173,174]. Recently, a RIA method has been developed to measure genistein levels in serum [175] and using fluoroimmunoassay for human urine assay [176]. Although the immunoassays are valuable methods, they are not yet commercially available, do not quantitate metabolites, and may lack specificity.

Recently, a simple isocratic LC–UV method in a phenyl column, using acetonitrile–water (33:67, v/v) as eluent was used to separate and quantitate genistein from soybeans [177]. In the plasma sample, an HPLC coupled with coulometric electrode array

Table 6
HPLC assay for harringtonine and homoharringtonine

Compound	Specimen	Column	Detection	Sample pretreatment	Reference
Harringtonine	Human plasma	RP-CN	ED	LLE	[140]
Homoharringtonine	Human plasma	RP-CN	ED	LLE	[140]
Harringtonine	Serum	Micro RP-C_{18}	Flu, Ex 280 nm, Em 320 nm	LLE	[138]

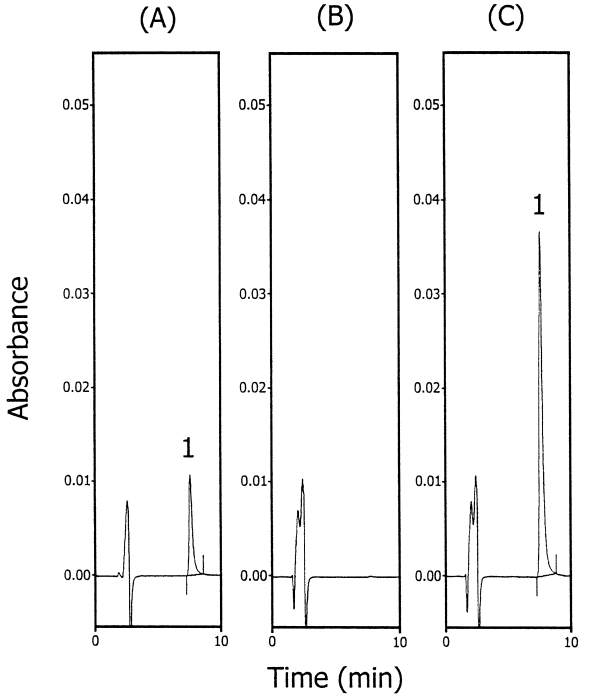


Fig. 12. (A) A standard injection of berberine (1 μg/ml). (B) A chromatogram of a blank blood dialysate. No peaks were observed that would interfere with the analysis of either compound. (C) A chromatogram of a blood dialysate sample containing berberine (3.48 μg/ml), which was obtained from blood microdialysis 20 min after berberine administration (20 mg/kg, i.v).

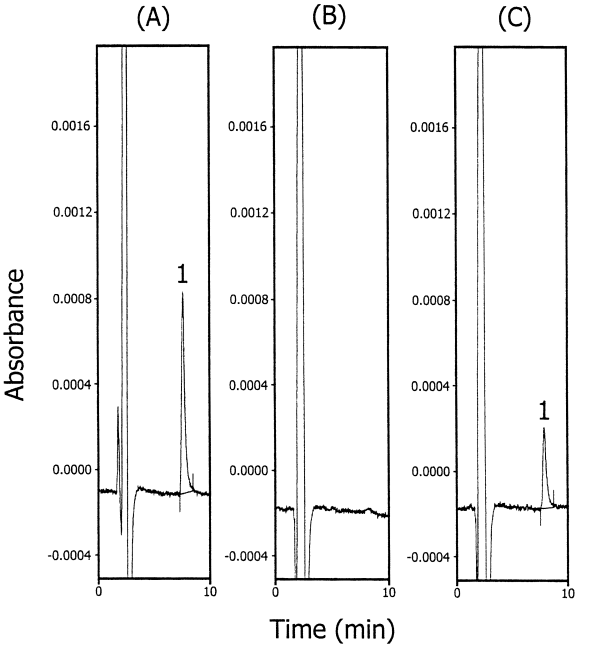


Fig. 13. (A) A standard injection of berberine (0.1 μg/ml), and (B) the chromatogram of a blank brain dialysate. (C) The chromatogram of a brain dialysate sample containing berberine (0.058 μg/ml) collected from a rat brain microdialysate 20 min after berberine administration (20 mg/kg, i.v.).

detection has been reported in detection limits at 40.3 (genistein) pg on column [170]. The described HPLC method for genistein assay in biological fluids is summarized in Table 9.

4. Miscellaneous

4.1. Berbamine

Berbamine (Fig. 17) is one of the important alkaloids present in the herb, *Berberis julianae* or *Berberis poiretii* (Chinese name: Shan-Ke-Zhen). This plant has been used in China folk medicine for the treatment of insomnia, fevers, inflammation and diarrhoea, as well as for prevention of rejection on skin transplants in mice [178]. Zhu and Sui [179] demonstrated that berbamine possesses significant anticancer activity [179,180]. Subsequently, it was suggested that berbamine may not only be useful as a leukogenic drug, but is also useful as an adjuvant in cancer chemotherapy and radiotherapy [181]. Recently, an HPLC–UV [182] method has been reported for the determination of berbamine from the stem woods of a Lauraceous plant, *Dehaasia triandra* Merr with a mobile phase of acetonitrile–0.05 *M* dihydrogen phosphate buffer (pH 3.0) (27:73, v/v) containing 0.1% diethylamine and 2 m*M* sodium heptanesulphonate. Sun and Wu [183] developed a micellar electrokinetic chromatographic (MEKC) method for the separation of berbamine from eight bisbenzylisoquinoline (BBI) alkaloids.

4.2. Daphnoretin

Daphnoretin (Fig. 18), a dicoumaryl ether, was isolated from the whole plant of *Daphne mezereum* and *Wikstroemia indica* C.A. Mey (Thymelaeaceae) (Chinese name: Nan-Ling-Jao-Hua) [184–186], which has been used in Chinese folk medicine as a

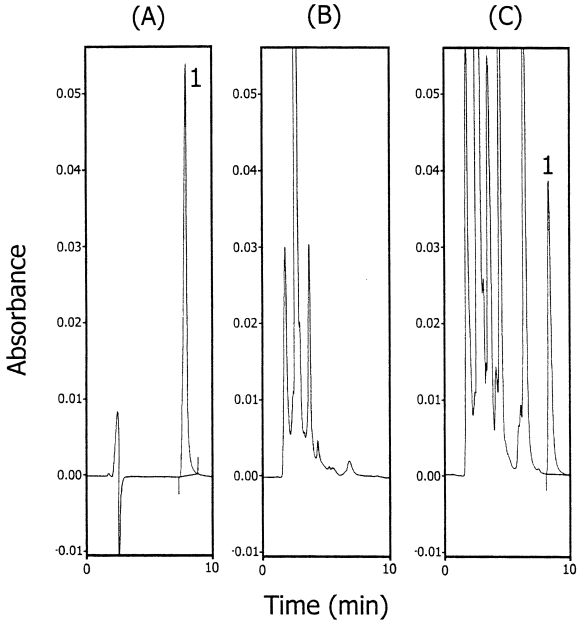


Fig. 14. (A) A standard injection of berberine (5 μg/ml). (B) A chromatogram of a blank bile dialysate sample obtained from bile duct microdialysis before the drug administration. (C) The chromatogram of bile dialysate sample obtained containing berberine (3.65 μg/ml) collected from the bile duct microdialysis 90 min after berberine administration (20 mg/kg, i.v.).

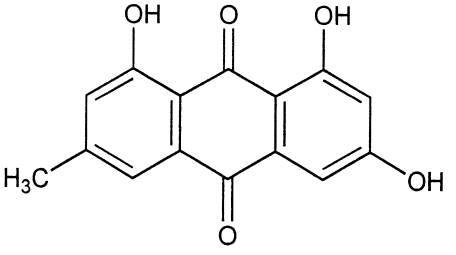


Fig. 15. Chemical structure of emodin.

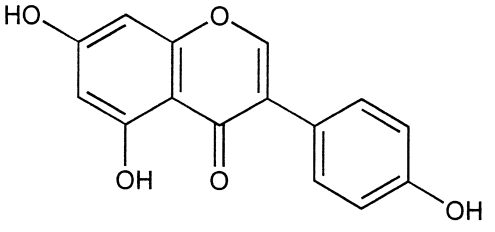


Fig. 16. Chemical structure of genistein.

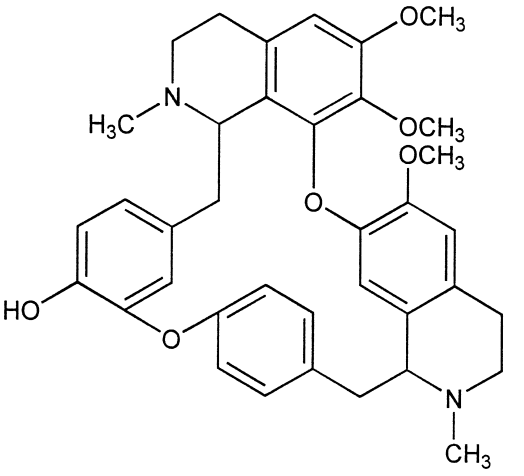


Fig. 17. Chemical structure of berbamine.

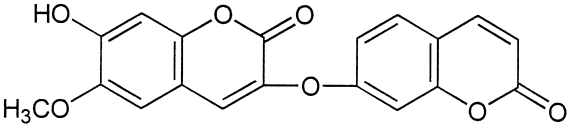


Fig. 18. Chemical structure of daphnoretin.

herbal remedy for the treatment of syphilis, arthritis, whooping cough [187], and cancer [188]. Daphnoretin has been demonstrated to significantly inhibit Ehrlich carcinoma growth, which inhibited leucine incorporation into protein by 77% after administration of the drug at 6 mg/kg per day for 3 days [185,186].

4.3. Irisquinone

Irisquinone (Fig. 19) was isolated from *Iridaceae latea pallasii* Fischer var. *chinensis* in the research for antineoplastic agent [131,189]. Experimental chemotherapeutic studies have shown that iris-

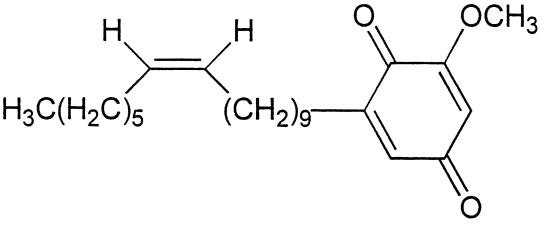


Fig. 19. Chemical structure of irisquinone.

Table 7
HPLC assay for berberine

Compound	Specimen	Column	Detection	Sample pretreatment	Reference
Berberine	Human plasma	μBondapak-C_{18}	UV 346 nm	LLE	[148]
Berberine	Rabbit plasma	RP-C_{18}	UV 267 nm	Protein precipitation	[149]
Berberine	Rabbit urine, bile	RP-C_{18}	UV 267 nm	LLE	[149]
Berberine	Human plasma	μBondapak-C_{18}	UV 346 nm	LLE	[150]
Berberine	Rat plasma	RP-C_{18}	UV 345 nm	LLE	[151]

Table 8
HPLC assay for emodin

Compound	Specimen	Column	Detection	Sample pretreatment	Reference
Emodin	Human urine	RP-C_{18}	Diode array	LLE	[158]
Emodin	Rabbit plasma	RP-C_{18}	UV 280 nm	Protein precipitation	[162]
Emodin	Rabbit plasma	LiChrosphere RP-C_{18}	UV 221 nm	Protein precipitation	[163]

Table 9
HPLC assay genistein

Compound	Specimen	Column	Detection	Sample pretreatment	Reference
Genistein	Rat serum	RP-C_{18}	LC–ES–MS	SPE	[171]
Genistein	Plasma, urine	Nona-Pak C_8	UV 260 nm	LLE	[168]
Genistein	Human urine	Bonded phase BP-1 capillary silica column	GC–MS	SPE	[166]
Genistein	Human urine	RP-C16	LC–APCI–MS	LLE	[172]

quinone is effective against transplantable tumors. It is also a sensitizer for radiation therapy of cancer [131,190]. To our knowledge, no analytical procedure has been described for irisquinone.

5. Conclusion

In summary, traditional Chinese medicine provides a valuable resource in the search for the new antineoplastic drugs. Coupled to various detection and sample preparation procedures, chromatographic methods are widely employed for herbal plants and their formulated preparations. The continuous search for new drugs from plants is an important direction in cancer treatment and chemoprevention.

6. Notation

APCI-MS, atmospheric pressure chemical ionization–mass spectrometry
CE, capillary electrophoresis
CZE, capillary zone electrophoresis
ED, electrochemical detection
Em, emission
ES–MS, electrospray–mass spectrometry
Ex, excitation
Flu, fluorescence
GC, gas chromatography
ISP-MS, ion spray mass spectrometry
LC, Liquid chromatography
LLE, liquid–liquid (phase) extraction
RIA, radioimmunoassay
SDS, sodium dodecyl sulphate

SPE, solid-phase (liquid–solid) extraction
TEAA, triethylamine acetate
UV, ultraviolet

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