CHROMATOGRAPHY OF ANTICANCER DRUGS. PART 3

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Various chromatographic techniques applied for the separation and quantitative determination of synthetic anticancer drugs and natural anticancer compounds are reviewed.

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Introduction

Various chromatographic techniques have been extensively applied not only for the separation and quantitative determination of synthetic anticancer drugs but also for the analysis of natural anticancer compounds present in complicated accompanying organic and inorganic matrices. The same chromatographic techniques were employed for the analysis of synthetic drugs as for the investigation of natural products. These methods have been reviewed.

Discussion

Liquid chromatography combined with tandem mass spectrometry (/LC/MS/MS/) was successfully employed for the separation and quantitative determination of doxorubicin and its main metabolite doxorubicinol (DOXol). Solid phase extraction was applied for the prepurification and preconcentration of samples, the validated calibration ranged from 5.00-to 1000 ng mL^{-1} and from 0.50 to 50.0 ng mL^{-1}. The data proved the the good accuracy and precisity of the method. It was established that the procedure can be used for the analysis of DOX and DOXol in whole cells, nuclear enriched fraction and organelle-enriched fraction. It was further found that the method is suitable for the determination of analytes in subcellular compartments.[1]

A HPLC-MS procedure was developed for the study of the efficacy of a non-hypercalcemic vitamin-D2 derived anti-cancer agent (MT19c). The measurements established the antitumor activity of the new preparation. It was stated that the compound shows marked antitumor effect and can be applied for the design of vitamin-D based anticancer molecules. It was further proposed for the developing of MT19c as a therapeutic agent for malignant ovarian tumors by targeting oncogenic de novo lipogenesis.[2]

An LC-MS method was developed for the investigation of the anticancer prodrug combretastatin A1 phosphate (OXi4503, CA1P), the active CA1 and its glucuronide metabolites in human urine and CA1 in plasma.

Validated methods were applied for the determination of CM1, the active agent derived from the prodrug CA1P, for the analysis of three glucuronides CA1G1, CA1G2 and CA1DG. Solid phase extraction was employed for the preconcentration of human plasma samples while urine samples were not pretreated. Validations were carried out in concentration ranges of 5 – 1000 nM (plasma CA1); 50 – 2000 nM (urine samples). The mean correlation coefficients were over 0.997. Mean recoveries were 101% (CA1 from plasma, 97% from urine), The measurements revealed the presence of two monoglucuronides and a diglucuronide.[3]

Optimization of the inclusion complex formation of the hydrophobic anticancer drug cifelin was studied in detail. It was established that the inclusion of the drug is stealth liposomes decreases toxicity and enhanced the circulation of the drug in the blood stream. The optimal composition of the liposome was found to be 165:8:1 w/w phosphatidylcholine:cholesterol. The composition of the liposomes was controlled by thin-layer chromatography (TLC) using butanol:glacial acetic acid:water (12:3:5, v/v) as mobile phase. The measurements indicated that the efficacy of inclusion complex formation was 98.3 %.[4]

Size exclusion chromatography (SEC) or dialysis were employed for the characterization of niosomal formulations of doxorubicin aimed to obtain a potential brain targeted delivery system. Formulations were functionalized with the glucose derivative N-palmitoylglucosamine (NPG). Various physicochemical methods such as light scattering, transmission electron microscopy, HPLC, this layer evaporation, were employed for the study of the characteristics of the doxorubicin formulations.The concentration of drug was determined in blood and various organs. It was established that the new formulation may help the better understanding of the mechanism of drug transport of functionalized niosomes.[5]

The influence of sodium thiosulfate (STS) on the side effect of the anticancer drug cisplatin (CP) was investigated in detail. Measurements were carried out by SEC combined with inductively coupled plasma atomic emission spectrometer (ICP-AES). It was found that the addition of STS modified considerably the decomposition rate of CP decreasing the side effect of CP. It was further suggested that similar measurements may help the decrease of side-effects of Pt based anticancer drugs.[6]

The influence of anticancer drug treatment on the protein map of pancreatic cancer cells was determined. Protein composition was analysed by two-dimensional (2-D), and nano-high performance liquid chromatography electrospray ionization time of flight mass spectrometry/mass spectrometry. It was stated that the distribution and concentration of protein fractions can be used for the detection and identification of protein fractions.[7]

LC-MS-MS was employed for the elucidation of the structure of the main unknown oxygenated metabolites of the new anticancer drug EAPB0203. The structure of metabolites were compared with those of synthetic standards. One- and two-dimensional H-1 NMR spectroscopies has also been applied for the elucidation of the structure of the new anticancer agent and its main metabolites.[8]

The separation and quantitative determination of the anticancer drug CYC in rat plasma was achieved by employing LC-MS methodology. Analyte was extracted from plasma samples using liquid-liquid extraction (ethyl acetate:water). Chromatographic analysis was carried out on a C18 column (150 mm x 4.6. mm i.d., particle size, 5 µm). The isocratic flow rate was set to 0.8 mL min^{-1}. Mobile phase consisted of acetonitrile-water-formic acid (23.5:76.5:0.1 v/v). The calibration curve of the drug was linear in the concentration range of 5-2.500 ng mL^{-1} (r = 0.9955). The main recovery ranged from 90.0 % to 110 %. The intra- and interday precisions were lower than 11.8 and 6.6 %, respectively. The accuracy of the method was within ±5.8 %. The investigations indicated that the method can be successfully applied for the study the pharmacokinetics of CYC-116 in rats after oral administration.[9]

A repeat dose study of the novel pro-apoptotic chemotherapeutic agent α-tocopheryloxyacetic acid (α-TEA) was assessed using male and female mices. It was established that α-TEA suppress tumor growth in various murine and human xenograft tumor models, including melanoma, breast, lung, prostate and ovarian cancers. Mice were treat with 100, 300, and 1500 mg kg^{-1} d^{-1} α–TEA. The serum levels were determined by LC-MS. No mortality was found, and no clinical signs of toxicity. Histopathological evaluation revealed no significant lesions. The half-life of orally administered α-TEA was determined as 52 h. It was stated that the results may facilitate the design of clinical trials to evaluate the safety and antitumor efficacy of α-TEA in patients with cancer.[10]

Bioreducible and core-cross-linked hybrid micelles were prepared from trimethoxysilyl-ended poly(e-caprolactone)-S-S-poly(ethylene oxide) bock copolymers. The structure of the novel copolymers were determined by various pysicochemical methods such as FTIR, 1H NMR, gel permeation chromatography, differential scanning calorimetry, wide-range X- ray diffraction, dynamic light scattering (DLS), transmission electron microscopy. It was proposed that the copolymers can be applied for the fabrication of bioreducible and core-crosslinked hybrid micelles potential for anticancer drug delivery system.[11]

The pharmacokinetics of 5-fluorouracil (5-FU) and cyclophosphamide (CP) in depression rats was investigated in detail. The effect of moode disorder on the drug metabolism process was assessed by the determination of the plasma drug concentration by HPLC for 5-FU and with HPLC-MS/MS for CP. The results revealed significant differences between the pharmacokinetic parameters of 5-FU and CP between in depression model rats and control group (p<0.05).[12]

A complex of cyclohexane-1,2-diaminoplatinum with an amphiphilic biodegradable polymer was prepared and applied as a drug carrier. The composition of the complex was analysed by HPLC combined with inductively coupled plasma mass spectrometry and X-ray photoelectron spectroscopy. It was stated that this novel complex may have a great potential application in clinical use.[13]

A new method was developed for the preparation of a drug loaded PLGA/PEVA composite (containing paclitaxel as model compound). It was established that the mixture of PLGA poly(lactide–co-glycolide) and PEVA (ethylene vinyl acetate) form an ideal carrier for paclitaxel (PTX). The morphology of the coating material was analysed by scanning electron microscopy, the release pattern of PTX was determined by HPLC.[14]

Fluorinated and pegylated polyaspartamide derivatives were prepared and employed to enhance the solubility and biological efficacy of flutamide The characteristics of the novel copolymers based on polyaspartamide were investigated by size exclusion chromatography, light scattering analysis, and scanning electron microscopy.[15]

Star-block copolymers consisting of a hyperbranched polyethyleneimine, a poly(t-glutamic acid) inner shell and a polyethylene outer shell were synthesized and characterized by H-1 NMR, GPC and TEM. It was found that the complexes showed relatively high temporal stability at physiological pH and the release of the encapsulated compounds decreased at higher pH values.[16]

A novel type of folic-acid (FA) based copolymers were synthesized and their characteristics were investigated by using H-1 NMR, GPS, TEM, DLS and confocal laser scanning microscopy. It was established that FA conjugated micelles could be excellent nanocarrier to deliver anticancer drugs specially inside the cell via FA –receptor-medicated endocytosis.[17]

The influence of process parameters on the co-precipitation of PTX and poly(L-lactic acid)was investigated by supercritical antisolvent process (SAS). The particle samples were characterized by XRD, SEM, HPLC, laser diffraction particle size analyzer. The results indicated that the solvent and the solvent ratio exert a marked influence on the particle morphologies The best operating conditions for the experimental system were as follows: DCM/EtOH 50/50, v/v; 35^0; 10-12 M Pa; PLLA, 5 g L^{-1}; solution flow rate 0.5 mL min^{-1}.[18]

A novel series of molecularly imprinted polymers (MIPs) based on acrylonitrile:methacrylic acid (AN:MAA) was synthesized and their characteristics were investigated by various physicochemical methods such as elemental analysis (EA), attenuated total reflectance infrared spectroscopy (ATR FT-IR), RAMAN spectroscopy, SEC, thermogravimetric analysis (TGA), DSC, and batch rebinding tests. It was concluded from the measurements that –COOH functional groups play a considerable role in the imprinting process. The target molecule was diosgenin an important anticancer and antileukemia compound.[19]

Preparation and optimization of media employing Pluronic micelles to enhance the solubilization of the drug sirolimus an antiinflammatory/antiproliferative and immunosupressive bioactive compound. The influence of the composition of the drug release medium was investigated in detail. The measurements indicated that the buffer composition (acetate or phosphate buffer) influenced considerably the behaviour of the drug sirolimus in aqueous environment. It was further found that the type and concentration of the micelles also influence the in-vitro release profile of sirolimus. It was further established that the critical micellization temperature (CMT), DLS, hydrodynamic size of micelles also influences the release profile of sirolimus.[20]

The impact of polyamine depletion of the anticancer activity of a trinuclear Pt-compound was determined. The polyamine concentration in the samples was reduced by adding α-difluoromethylornithine (DFMO) or N-1,N-11-diethylnorspermine (DENSPM) to the samples. The anticancer activity of the drug was determined by HPLC analysis can increase the toxicity The results suggested that the combination of polyamine synthesis inhibitors with trinuclear Pt compound can enhance the antitumor activity of the trinuclear compound. It was assumed that the combination of polyamine synthesis inhibitors with trinuclear anticancer drug increases the toxicity of a trinuclear Pt compound.[21]

Liquid chromatography combined with tandem mass spectrometry was employed for the analysis of XMT-1001, a novel, polymeric topoisomerase I inhibitor. HPLC was applied for the determination of XMT-1001, conjugate release products, CPT-20-O-N-succinidimido-glycinate; CPT-SI and CPT-20-O-N-succinilamido glycinate. It was established that conjugated drug shows enhanced antitumor efficacy compared macromolecular camptothecin drug conjugate.[22]

Novel amphiphilic, biodegradable, and biocompatible cross-linked copolymers were synthesed and the drug deliver capacity was assessed. Copolymers were prepared with 2-methylene-1,3-dioxypane (MDO), poly(ethylene glycol)methylether methacrylate (PEGMA) and 7-(2-metacryloyloethoxy)-4-methylcoumarin metacrylate (CMA). The copolymers were investigated by H-1-NMR, C13 NMR, GPC. DLS, and TEM. The hydrolytic degradation and enzymatic decomposition of the polymers were also determined. DOX was employed as target compound. The measurements indicated that these new copolymers can serve as promising nanocarriers for the delivery of anticancer drugs.[23]

Another anticancer drug delivery system was developed applying lactosyl-norcantharidin-associated N-trimethyl-chitosan nanoparticles (Lac-NCTD). The concentration of the drug in the samples were determined by HPLC. It was concluded from the data that the concentration of the anticancer drug in the samples depended on the temperature, pH value of the environment and the composition of the anticancer drug delivery system. It was further found that the polymer can penetrate the plasma membrane of CaCo-2 cells.[24]

The synthesis and biochemical characterization of a novel multifunctional biopolymer was reported. The drug delivery system contained the anticancer drugs daunorubicin and methotrexate. GnRH-III decapeptide served as targeting moiety. Bioconjugates were prepared from amino acids: [(4)Lys]-GnRH-III (Glp-His-Trp-Lys-His-Asp-Trp-Lys-Pro-Gly-NH_2). The concentration of anticancer drugs in the samples were determined by LC-MS. It was established that the biological efficacy of the preparation containing two anticancer drugs was higher then those containing only one anticancer drug.[25]

A novel drug delivery system was developed, characterized and its characteristics were investigated in detail. The novel ampiphilic graft polymer was prepared by using poly lactic acid and monomethyl polyethylene glycol. The characteristics of the novel copolymers were determined by 1-H NMR, FTIR, GPC, TEM, DLS, and CMCs The anticancer drug DOX was loaded into the micelles. The measurements suggested that the anticancer efficacy of the DOC loaded preparation was higher than those of free drug. It was further established that these micelles can be employed as promising potential carriers for delivering anticancer drugs.[26]

Multiarm poly(acrylic) star polymer was prepared and applied in sustained delivery of cisplatin and a nitrogen oxide prodrug. The product showed excellent water solubility and markedly low viscosity. The hydrophilic drug cisplatin and a hydrophobic nitric oxide was selected as model compounds. It was concluded from the results that the multiarm poly(acrylic acid star) polymer is suitable for the sustained release of cisplatin and a nitrogen oxid product.[27]

Micellar electrokinetic chromatography-laser-induced fluorescence method (MEKC-LIF) was employed for the analysis of DOX in biological samples . The migration buffer of the system consisted of 10 mM borate, 100 mM sodium dodecyl sulfate (SDS) (pH 9.3). Responses were linear in the range of 11.3-725 ng mL^{-1}; limit of quantitation (LOQ) was 43.1 ng mL^{-1}, limit of detection LOD) was 6.36 ng mL^{-1}. It was stated that the MEKC-LIF method can be applied as a powerful diagnostic tool for monitoring the intracellular DOX distribution influencing cytotoxicity.[28]

The correlation between the chromatographic behaviour and antitumor activity of curcuminoids was investigated by using high performance liquid chromatography-electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS). The data were evaluated by the method of orthogonal partial least squares (OPLS) and by canonical correlation analysis (CCA). The method was proposed for the discovery of antitumor active constituents.[29]

A validated HPLC method was developed for the determination of the encapsulation efficacy of curcumin in poly(lactic-co-glycolic acid)) (PLGA) and poly(lactic-co-glycolic acid) - polyethylene glycol (PLGA-PEG) nanoparticles. HPLC analyses were carried out under reversed phase conditions using C18 column (250 mm x 4.6.mm, 5 µm particle size). Isocratic mobile phase consisted of ethanol:water:acetonitrile (v/v/v) at the flow rate of 0.8 mL min^{-1}. The excitation and emission wavelengths were 365 and 512 nm, respectively. The selectivity, linearity, precision, accuracy, robustness, LOD and LOQ were determined. LOD and LOQ values were 9.65 and 50 ng mL^{-1}. The intra-and inter-assay coefficients of variation were less than 3.73 %. The maximum relative

standard deviation was 3.08 %. The data indicated that the method can be successfully applied for the determination or curcumin in PLGA and PLGA-PEG nanoparticles[30]

The antitumor activity of free and nanosponge-encapsulated camptothecin was investigated using human prostate cancer cells as model compounds. The objectives of the measurements were the enhancement of the poor water solubility of the anticancer drug. It was found that the activity of β-cyclodextrin nanosponge encapsulated camptothecin showed higher anticancer activity. HPLC measurements indicated that the biological efficacy of the bioactive anticancer drug is higher in encapsulated form.[31]

Integrated rapid resolution LC-MS was employed for the screening and identification of the metabolites of the potential anticancer agent 3,6,7-trimethoxyphenanth-roindolizidine (CAT) in rat urine. Analyses were performed by employing combination of multi-period product ion-scan (mpMS/MS) with high resolution characteristic extracted ion chromatograms. It was further found that the method allowed the separation and identification of 21 metabolites and the determination of the structure of 9 metabolites.[32]

cDNA cloning, overexpression, purification and pharmacological evaluation for anticancer activity of ribosomal protein L23A gene (RPL23A) from giant panda (*Alluropoda melanoleuca*) was performed. The expression product was further purified by Ni chelating affinity chromatography.[33]

Capillary electrophoresis technologies are frequently used in the analysis of various organic and inorganic components present in complicated accompanying matrices. The newest methods has been recently discussed including the separation and quantitative determination of a wide variety of analytes such as impurity profiling, quality control, quality control of pharmaceutical formulations, lipophilicity determination, interaction between metallodrugs and proteins or nucleotides, characterization and quantification of metabolites in biological matrices and real-world samples, etc.[34]

The pharmacokinetic of the complex EAK-EPT was investigated in detail (EAK amino acid pairing peptide), (EPT anticancer agent). The measurements was carried out by HPLC, and indicated that EAK can serve as a suitable carrier to increase the bioavailability of EPT.[35]

The differences between the physical properties of the inner and outer leaflet of membranes was elucidated by using a combined chromatography/cyclodextrin procedure suitable for the selective labelling of outer and inner leaflet. It was assumed that selective labelling influence the curvature of the membrane.[36]

The synthesis of a new kind of amphiphilic, biodegradable, biocompatible, cross-linkable copolymers was reported and their application for drug delivery was elucidated. Copolymers were characterized by H-1 NMR, C-13 NMR, DLS, TEM and GPC. The capacity of the polymers to deliver the anticancer drug doxorubicin was also investigated. The measurements indicated that the composition of polymers exerts a marked influence on the drug release behaviour. It was assumed that these novel copolymers can serve as promising nanocarriers for the delivery of anticancer drugs.[37]

The antimicrobial activity of essential oils (EO) against Streptococcus mutant was investigated in detail. Twenty EO were included in the experiments. Active ingredients were achieved by hydrodistillation and chemical methods. The minimum inhibitory concentration (MIC) and bactericidal (MBC) were also determined. Chemical analyses were carried out by employing thin-layer chromatography and gas chromatography/mass spectrometry. The data indicated that some fractions of EO contained fractions with marked antiproliferative effect.[38]

A novel class of anticancer prodrugs were prepared and experimentally applied. Styryl conjugated 2-nitrobenzyl derivatives were introduced as phototrigger to reduce the drug release. Chlorambucil was employed as model compound. The drug release was followed by measuring UV-vis absorption, FT-IR, and HPLC spectra. It was further established that the release of chlorambucil can be regulated by the modification of external light condition.[39]

The chemopreventive activities of 3,6-dihydroxyflavone (3,6-DHF) against mammary carcinogenesis was studied in detail. The bioavailability of 3,6-DHF in rats was determined by HPLC. The results indicated that the oral administration of 3,6-DHF supressed the breast carcinogenesis induced 1-methyl-1-nitrosourea (MNU). It was found that 3,6-DHF decreased the cancer incidence by 35.75 %. It was concluded from the results that 3,6-DHF is a potent natural chemopreventive agent influencing the anticancer mechanism of flavonoids.[40]

The stability of 5-fluorouracil a chemotherapeutic agent was investigated under different conditions. HPLC and infrared spectroscopy were applied for the separation and quantitative determination of the analytes. HPLC measurements were carried out on a C18 column using 40 mM KH_2PO_4 mobile phase. The analytes were detected at 260 nm wavelength. The correlation coefficient was 0.9995. The R.S.D. values for intra-day and inter-day precisions were lower than 0.2 % and 1 %, respectively. It was established that the drug is not stable under alkaline conditions, but stable when exposed to UV irradiations.[41]

The analytical methods used for the determination of metallodrugs have been previously reviewed. The advantages and disadvantages of the various up-to date separation technologies have been enumerated and discussed in detail. The applicability of ICP-MS (inductively coupled plasma mass spectrometry) ICP-MS in the various field of the analysis of metallodrugs in biological samples.[42]

The characterization of recombinant human IL-15 deamidation was followed by RP-HPLC/ESI-MS measurements. It was found that the deamidation rate depended considerably on the pH value of the mobile phase, on the temperature and composition of the solvent phase.[43]

HPLC-UV method was employed for the study of the chemical composition of acetone extract of the lichens *Parmelia caperata, P. saxatilis* and *P. sulcata*. The antioxidant, antimicrobial and anticancer activities of the

main metabolites were also investigated. It was established that the main phenolic compounds in the extracts were protocetraric and usnic acid (*P. caperata*) and depsidone salazinic acid. Moreover, some samples contained atranorin and chloroatranorin. The investigations demonstrated the marked antioxidant, antimicrobial and anticancer activity of some extracts. The results indicated that these lichens can be applied as new sources of natural antimicrobial agents, antioxidants, and anticancer compounds.[44]

The efficacy of various chromatographic technologies for the separation and quantitative determination of proteins was compared. It was found that SDS-PAGE is not suitable for this type of analysis. The results indicated that high performance size exclusion chromatography (HP-SEC), strong anion exchange (SAX), weak cation exchange (WCX) can be applied for the analysis of ovalbumin, myoglobin, and bovine serum albumin (BSA). The RSD values (peak areas day-to-day) were similar for each stationary phase: SEC<1.9 %; SAX>5 %; RP>2 %; WCX<3.5 %. The analysis of an IgG1 type antibody was also included in the experiments.[45]

The presence of bioactive peptides in marine organism, the methods for their separation and purification using different chromatographic technologies have been previously discussed. Peptides with antimicrobial, antitumoral and antiviral activity were discovered and isolated. Various phyla such as *Porifera, Cnidaria, Nemertina, Crustacea, Mullusca, Echinodermata,* and *Cramata* were investigated for their biological activity.[46]

HPLC combined with mass spectrometry was employed for the affinity screening of bioactive component from herb medicine. The bioactive compounds paclitaxel, resveratrol, ketoprofen an penicillin G were included in the investigation. It was established that the three-dimensional cell bioreactor coupled with HPLC/MS can be successfully applied for affinity screening and analysis of bioactive components interacting with cells.[47]

HPLC-MS was employed for the separation and quantitative determination of the anticancer prodrug combretatstatine A1 phosphate (OX4503, CAIP) active CA1, and its glucuronide metabolites in human urine and of CA1 in plasma. Solid phase extraction was applied for the preconcentration of CA1 from plasma, while urine samples were analysed without pretreatment. Assays were validated between 50-2000 nM (CAIP), 25-2000 nM (CAI), 50-40.000 nM CA1G1 and CA1G2, 25-4000 CAIDG. Main recoveries varied between 92 and 101 %.[48]

The mass balance, excretion and metabolism of [C-14] ASA404 was investigated in cancer patients in a phase I trial. Measurements were carried out by HPLC. ASA404 was involved in the investigation because of its tumour vascular discrupting capacity. It was established that the method identified two novel metabolites not detected with other methods.[49]

A specific and sensitive enzyme-linked immunosorbent assay (ELISA) was developed for the pharmacokinetic studies of vindesine (VDS). The results were compared with those measured by HPLC. It was found that the results obtained by ELISA and HPLC were commensurable. It was further established that the method showed a very weak cross-reactivity with other vinca alkaloids such as vincristine (0.18 %) and vinblastine (0.11 %). The measurements proved that the sensitivity of ELISA method was 50-fold higher then the HPLC procedure. It was assumed that the ELISA procedure can be successfully used for the pharmacokinetic studies of VDS.[50]

The efficacy of quercetin and liposomal quercetin was compared using PEGylated nanomaterials containing polyethyleneglycol-2000-distearoyl phospatidylethanolamine. The data were evaluated by TEM and HPLC/UV spectroscopy. The investigations indicated that liposomal formulation of quercetin is more effective drug delivery vehicle in vivo as tumor-targeted drug carriers.[51]

It is well known that the low water solubility and bioavailability limits the application of curcumin in clinical practice. A new nanoparticle curcumin preparation was developed and its pharmacokinetics and safety was investigated employing various physicochemical and biophysical methods such as HPLC. It was further assumed that the novel preparation can improve the bioavailability of curcumin in human subjects.[52]

The antitumor and angiostatic activity of frog skin excretions of *Phyllomedusa bicolor* (South American tree frog) was investigated. The crude skin exudate was further purified by SEC and HPLC. The measurements indicated that two peptides belonging to the dermaseptin family were responsive for the antitumor and angiostatic activity. It was stated that this compounds can be used for the development of novel class of anticancer drugs.[53]

The anticancer and immunostimulator activity of the conjugate of paclitaxel and a non-toxic derivative of LPS was investigated by HPLC, NMR and IR. It was established that the stability of the preparation depended considerably on the pH and temperature. It was further found that the conjugate exhibited chemotherapeutic and immunotherapeutic activity in vitro. It was concluded from the results that this conjugate is a potential chemo-immunotherapeutic preparation showing high anticancer activity, and less toxicity and easy of delivery.[54]

The influence of betulin enriched birch extracts on human carcinoma cells and ear inflammation was investigated in detail. A novel more effective extraction procedure was developed and applied for the analysis of the active components in the samples. The extracts were further investigated by HPLC-MS, Raman, SERS and C-13 NMR. The antiviral activity of the extracts were determined on skin epidermoid carcinoma, ovarian carcinoma, cervix adenocarcinoma, and breast adenocarcinoma. Each extract showed marked anticancer activity. The measurements further indicated that each extract contain considerable antiproliferative and anti-inflammatory activity too.[55]

RP-HPLC method was developed for the analysis of camptothecin (CPT) incorporated into solid nanoparticles (SLN). The concentration of CPT in some rat organs (brain, heart, kidneys, liver, lung, spleen) was determined. The temperature of separation was 30 ^{0}C. Analytes were separated by gradient elution, mobile phase consisted of triethylamine buffer pH 5.5 and acetonitrile at a flow-rate

1.2 mL min^{-1}. Running time was 16 min. Analytes were detected by fluorometric method the excitation and emission wavelength being 360 and 440 nm. The calibration curves were linear in each case (r>0.9999) between 1-200. It was stated that the new method is reliable, precise, accurate and suitable for the analysis of CPT in rat organ samples in physical mixture with SLN, and incorporated in SLN.[56]

Because of their cancer preventive activity the lipophilic compounds of wheat bran were extensively investigated employing HPLC technologies. It was established that fractions containing unsaturated free fatty acid, phytosteroids, and alkylresorcinols showed high cytotoxic activity. The anticancer effect of the pure fractions was determined on human prostate adenocarcinoma (PC3) cells. It was further established that pure compounds 5-heptadecylresorcinol (IC50=22.5 µg mL^{-1}); 5-(16-heneicosenyl)resorcinol (trans) (IC50 = 13.7 µg mL^{-1}; 5-(14-nonadecenyl)resorcinol) (trans) IC50 = 42.2 µg mL^{-1}; 5-(2-oxoticosanyl)resorcinol) (IC50 = 10.9 µml) showed marked anticancer activity. It was concluded from the results that alkylresorcinols are important in the cancer preventive activity of wheat bran. It was further concluded from the data that other components such as free fatty acids and phytosterols also influence the anticancer activity of wheat bran.[57]

MEKC-LIF technique (micellar electrokinetic chromatography) combined with laser-induced fluorescence was employed for the investigation of the subcellular localization of DOX in biological samples. The migration buffer consisted of 10 mM borate, 100 mM SDS sodium dodecyl sulfate (SDS) (pH 9.3). The correlation between the chromatographic parameters and the concentration of the analyte in the mobile phase was linear between 11.3-725, the limit of quantitation (LOQ) was 43.1 mg mL^{-1}; the limit of detection (LOD) was 6.36 ng mL^{-1}. The measurements indicated that liposomal carriers enhance the efficiency of liposomal carrier in delivering DOX into the nucleus. It was established that subcellular fractionation followed by liquid-liquid extraction and MEKC-LIF. The method was proposed for the investigation of the intracellular distribution of DOX.[58]

A GC/MS method was developed and successfully applied for the analysis of the components of Flammulina velutipes (FVS), a potential antitumor agent. The objectives of the investigation was the determination of the growth inhibition activity of FVS against certain human cancer cell lines (gastric SGC and colon LoVo) and the study of the pharmacokinetics of encapsulated FVS. The components separated and quantitavely determined were: ergosterol (54.8 %), and 22,23-dihydroergosterol (27.9 %). The measurements indicated that the preparation showed strong in vitro proliferative activity against SGC cells. It was concluded from the results that FVS can be a possible candidate for the development of an anticancer drug preparation. Using microemulsion formulation FVS can be applied for the development of bioavailable preparations.[59]

Liquid chromatography/radiodetection/mass spectrometry was employed for the preclinical evaluation of the metabolism and disposition of RRx-001, a novel anticancer agent. Investigations revealed the presence of four main metabolites.[60]

Supercritical carbon dioxide followed with LC was applied for the extraction of ar-turmerone (aromatic volatile turmeric oil from Curcuma longa Linn). It was established that aromatic turmerone showed marked anticancer activity with 50 % inhibitory concentrations of 64.8±7.1; 102.5±12.5 and 122.7.6 against HepG2, Huh-7 and Hep3B cells. The data suggested that ar-turmerone deserves further investigations as a natural anticancer and cancer-preventive agent.[61]

The metabolic profile of the anticancer drug panobinostat was determined by LC followed with radiometric detection and LC-tandem mass spectrometry. Radioactivity was recovered after 7 days (44-77% in feces and 29.51% in urine). The results indicated that panabinostat and its metabolites were excreted in similar amounts through the kidneys and liver with good dose of recovery.[62]

The mutagenic and antimutagenic activity of the methanol leaf extract of Myristica fragrans was investigatid by both in vivo and in vitro methods. Gas chromatogtaphy/mass spectrometry was employed for the separation and quantitative determination of phytochemicals. It was assumed that phytochemical compounds with antioxidant activity may be responsible for the biological activity.[63]

The metabolism and accumulation of the lipophilic deoxinucleoside analogues cytarabine and gemcitabine was investigated by using TLC and HPLC. The results suggested that these compounds are suitable for novel clinical applications.[64]

A new LC-MS/MS analytical method was developed and validated for the separation and quantitative determination of seven anticancer drugs (cyclophosphamide, ifosfamide, irinotecan, etoposide, gemeitabine, carboplatin and pemetrexed) in human plasma. Analytes were extracted with two different methods and separated on a C18 column (2.1 mm x 100 mm x 3 µm particle size) with gradient elution. Positive electrospray ionization was used as ionization source. The mobile phase consisted of acetonitrile-water (0.1% formic acid and 10 mM ammonium acetate). Flow rate was set to 0.2 mL min^{-1}. Linear correlation coeffients were >0.992 for each anticancer drug. The accuracy was ±10.5 %. The mean recovery ranged from 50.0 to 81.0 %. The method was successfully applied to clinical samples of cancer patients.[65]

The application of GC with electron capture detection, GC-MS and HPLC for the analysis of clioquinol (5-chloro-7-iodo-8-quinolinol) has been previously discussed. The mechanism of action and the clinical uses in neurodegenerative disorders have also been reviewed.[66]

The conjugation of anticancer drugs through endogenous monoclonal antibody cysteine residues has been discussed in detail. It was stated that the conjugates can be readily analyzed by HPLC methods.[67]

The synthesis of podophyllotoxin, by an endophytic fungus Fusarium solani was previously reported and the its separation and quantitative determination by HPLC was achieved.[68]

The anti-tumor activity of the methanolic extract of Salvia mentifolia was investigated. It was established that all the organs showed anti-tumor activity. HPLC measurements proved the presence of polyphenols. Rosmarinic acid, caffeic acid, luteolin-7-0-glucoside and quercitrin were present in each sample. The measurements indicated that genus Salvia is a natural source of anti-tumor agents, however, the amount of anticancer agents show considerable differences.[69]

The influence of depression on the pharmacokinetic of 5-fluorouracil (5-FU) and cyclophosphamide (CP) was investigated using female Sprague-Dawley rats. The concentration of anticancer drugs were followed by HPLC-MS/MS. It was concluded from the measurements that depression mode disorder might alter drug metabolism process.[70]

Abbreviations

AN	acrylonitrile
CCA	canonical correlation analysis
CMT	critical micellization temperature
CMCs	critical micelle concentration
CP	cisplatin
CPT	camptotechin
CP	cyclophosphamide
3,6-DHF	3,6-dihydroxyflavone
DLS	dynamic light scattering
DOX	doxorubicin
DOX ol	doxorubicinol
EA	elemental analysis
ATR FT-IR	attenuated total reflectance infrared spectroscopy
5-FU	5-fluorouracil
GPC	gel permeation chromatography
HP-SEC	high performance size exclusion chromatography
ICP-AES	inductively coupled plasma atomic emission spectrometer
LC/MS/MS	liquid chromatography tandem-mass spectrometry
LOD	limit of detection
LOQ	limit of quantitation
Maa	methacrylic acid
MNU	1-methyl-1-nitrosourea
NPG	N-palmitoylglucosamine
MEKC-LIF	micellar electrokinetic chromatography-laser-induced fluorescence
MBC	minimum bactericidal concentration
MIPs	molecularly imprinted polymers
OPLS	orthogonal partial least squares
PEVA	ethylene vinyl acetate
PLGA	poly(lactide-co-glycolide)
PTX	paclitaxel
RP-HPLC	reversed phase high performance liquid chromatography
SAX	strong anion exchange
SDS	sodium dodecyl sulfate
SEC	size exclusion chromatography
SLN	solid lipid nanoparticles
STS	sodium thiosulfate
AS	supercritical antisolvent process
TLC	thin layer chromatography
TEM	transmission electron microscopy
TGA	thermogravimetrical analysis
WCX	weak cation exchange

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