CHROMATOGRAPHY OF ANTICANCER DRUGS

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The objectives of the series of reviews are the collection, concise description, comparison and evaluation of the various chromatographic methodologies applied for the separation and quantitative determination of anticancer drugs in various accompanying matrices.

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Introduction

Chromatographic techniques have been developed and successfully applied for the analysis of a considerable of number of organic and inorganic compounds present at trace level in sometimes complicated accompanying matrices. The rapid development of new chromatographic instrumentation and separation strategies resulted in the increase of the number of chromatographic technologies with higher separation capacity. Because of their considerable importance in human health care a high number of synthetic and natural compounds were investigated for possible anticancer activity. The different character of synthetic and natural products made necessary the separated discussion of the chromatographic techniques applyed for the investigation.

Chromatographic investigation of synthetic anticancer drugs

Liquid chromatography (LC) of synthetic anticancer drugs: Because of their high separation capacity various liquid chromatographic (LC) methods such as normal and reversed phase performance liquid chromatography (RP-HPLC), ultra performance liquid chromatography (UPLC), size exclusion chromatography (SEC), gel permeation chromatography (GPC) have been frequently employed for the analysis of synthetic anticancer drugs and for their formulation. The oxidative damage to guanine nucleosides following combination chemotherapy with 5-fluorouracil and oxiplatin has been recently investigated using UPLC. It was established that chemotherapy increases the concentration of metabolites. It was further established, that smoking, sex, and age exerts a marked influence on the oxidative damage.[1]

The analytical methods used for the determination of metallodrugs in biological samples have been recently collected and critically evaluated. Future trends have also been discussed in detail.[2] The stability of 5-fluorouracil a widely used chemotherapeutic agent has been investigated under various conditions using HPLC and infrared spectroscopy (IR). HPLC measurements were carried out on a C18 reversed phase column the mobile phase consisted of 40 mM KH_2PO_4. Analyted were detected at 260 nm. The relationship between the detector response and the concentration of the analyte was linear between 20 and 550 μg mL^{-1}. The coefficient of correlation was 0.9995. The relative RSD values of intra-day and inter-day were >0.2% and >1%. Investigations indicated that the decomposition rate of the analyte was higher in alkaline then in acidic conditions. UV radiation exerts a minimal influence on the stability of the drug.[3] The effect of adryamycin treatment on the mouse splenic protein levels was studied in detail using reversed-phase liquid-chromatography-tandem mass spectrometry. The measurements indicated that the drug exerts a marked effect of the protein levels.[4] The application of thermochemotherapy mediated by a new solar planet-structured magnetic nanocomposites has been recently reported. Docetaxel (an anticancer drug) was encapsulated and the efficacy of encapsulation and the drug release was followed by HPLC. It was stated that the method can be employed as an effective mediator for magnetic thermochemotherapy.[5] A new, specific and sensitive LC-MS/MS analytical method was developed for the simultaneous determination of eleven thiopurine nucleotides. Synthetic products such as eleven mono-, di- and triphosphates of thioguanosine, methylthioinosine, methylthioinosine, thioinosine, and methylthioguanosine. It was further established that the RSD value was below 8%, the recovery varied between 92% and 107%. It was further found that the method can be successfully employed for the better understanding of thiopurine metabolism.[6]

The behaviour of the anticancer drug doxorubicin (DOX) and it loaded form with poly(alkylcyanoacrylate) nanoparticles was studied by using HPLC technology. The method made possible the extraction of Dox and its metabolites by liquid-liquid extraction. The efficacy of extraction of total Dox (free Dox and Dox associated with nanoparticles) was 71 and 78 %. It was concluded from the data, that the within-day and between day precisions of the method were 97.1 – 102.9% and 97.3 – 101.7%. It was further suggested that the method is suitable for the determination the pharmakokinetic and biodistribution of DOX associated with nanopaticles.[7] Both SEC and HPLC were employed for the development and characterization of a niosomal formulation of doxorubicin aimed at brain targeting. Loaded and unloaded niosomes were separated by SEC. It was stated that niosomal formulations improve doxorubicin brain delivery.[8] Doxorubicin-loaded Fe_3O_4 magnetic nanoparticles were prepared and modified with biocompatible copolymers. The anticancer drug doxorubicin hydrochloride was encapsulated into poly (D,L-lactic co-

glycolic acid) poly(ethylene glycol) (PLGA-PEG). The copolymers were characterized with ^{1}H NMR spectroscopy, gel permeation chromatography, Fourier transform infrared spectroscopy, differential scanning calorimetry, X-ray powder diffraction, scanning electron microscopy and vibrating sample magnetometry. The drug encapsulation efficacy were 69.5%, 73% and 78% for PLGA, PEG(2000), PLGA, PED(3000) and PLGY PEG(4000), respectively. It was proposed that these nanoparticles can be employed for biomedical investigations.[9] A LC/MS/MS method was developed and successfully applied for the determination of doxorubicin and its primary metabolite (doxorubicinol) in cultured human leukemia cells. Solid phase extraction was employed for the clean up and enrichment of analytes. The calibration range was 5.00-1000 mg mL^{-1} and 0.50-50 ng mL^{-1} for doxorubicin and its metabolite, respectively. It was established that the anticancer drug and its primary metabolite can be simultaneously determined by LC/MS/MS.[1]

A new type of alendronate conjugated amphiphilic hyperbranched polymer based on Boltom H-40 and poly(ethylene glycol) for bone targeted drug delivery was synthesized. The star copolymer was investigated by FTIR and GPC. The investigations indicated that this type of micelles are highly promising bone-targeted drug carriers for skeletal metastases.[11] Dendron-like poly(ε-benzyloxycarbonyl-L-lysine)/linear PEO block copolymers were synthesized and their behaviour under various conditions was investigated. The physicochemical characteristics were investigated by FTIR, ^{1}H-NMR, GPC, differential scanning calorimetry, polarized, optical microscopy, and wide range X-ray.[12]

A new type of cyclodextrin-overhanging hyperbranched core-couble-shell miktoarm architectures were developed. The polymers were characterized by 1H-NMR, FTIR, SEC.[13] Affinity chromatography was employed for the determination of the effect of spectrin α II and β II tetramers on the platinum anticancer drug resistance in ovarian serous adenocarcinoma. The measurements indicated that proteins Spectrin α II and β II may contribute to drug resistance.[14] The effect of sodium thiosulphate on the metabolism of cisplatin in human plasma has been investigated by using size exclusion chromatography coupled to an inductively coupled plasma atomic emission spectrometer. It was established that the results can be used to develop feasible strategies to reduce the side effects of Pt-based anticancer drugs in patients.[15]

Biocompatible amphiphilic block copolymers with various molecular weight were synthesized and applied for the development of anticancer drug nanocarriers. The new copolymers were characterized by nuclear magnetic resonance and gel permeation chromatography. It was established that the new copolymers can be employed for the development of drug delivery systems with various molecular weight and with drug various drug loading capacity.[16] The anticancer drug methotrexate (MTX) was incorporated in layered ceramic nanoparticles of layered double hydroxides (LDHs). The nanoassemblies showed marked elevated chemical and thermal stability as detemined with HPLC measurements. It was assumed that the high cellular uptake may be due to the enhanced drug efficacy.[17] Pharmacokinetics of the peptide mediated delivery on the anticancer drug ellipticine has been investigated in detail. The complex of the amino acid pairing peptide (EAK16II) with the hydrophobic anticancer agent ellipticine was prepared and the pharmacokinetics parameters were determined by HPLC. The measurements indicated that EAK prolongs the residence time of the drug and increases the bioavailability.[18] The synthesis and characterization of poly(ethylene glycol)-b-poly(epsilon-caprolactone) copolymers with functional side groups on the polyester block was reported. The new polymers were characterized with differential scanning calorimetry, 1H NMR, GPC, fluorescence spectroscopy, transmission electron microscopy, and dynamic light scattering. The weak drug entrapment efficacy and drug-loading ability of these copolymers were established.[19]

The oral bioavailability and gender-related pharmacokinetics of celastrol, an anti-inflammatory and antitumor agent was investigated by LC/MS/MS. The measurements indicated that the LC/MS/MS method was selective, and precise. It was further found that formulation markedly increased the bioavailability, and female rats showed significantly better absorption then males.[20] Surface modification of paclitaxel-loaded polymeric nanoparticles was achieved and the in vitro cellular behaviour and in vivo pharmacokinetics was studied in detail. Samples were analysed with atomic force microscopy, dynamic light scattering, and cell confocal microscopy. It was concluded from the results that the surface modification of polymeric nanoparticles results in modified pharmacokinetik behavior.[21]

LC/MS/MS technology was employed for the simultaneous determination of seven commonly used anticancer drugs. The anticancer agents included in the investigations were: cyclophosphamide, ifosfamide, irinotecan, eloposide, gemcitabine, carboplatin and permetrexet. The separation of analytes was carried out on a C18 column (2.1 mm x 100 mm), 3 mm particle size. Positive electrospray ionization was employed as the ionization source. Mobile phase consisted of acetone-water (0.1% formic acid and 10 mM ammonium acetate). The flow rate ranged 0.1 – 0.25 mL min^{-1}. Linear coefficients were always over 0.992. The recovery ranged from 50 to 81.0%. The method was successfully applied to samples from cancer patients.[22] An LC-MS method and radiometric detection was developed for the study of the disposition, metabolism, and excretion of the orally active pandeacetylase inhibitor in cancer patients. Recovery of radioactivity in excreta was 87% (44 – 77% in feces and 29–51% in urine). Approximately 40 metabolites were detected, in was found that biotransformation begins at the hydroxamic acid side chain and ethyl-methyl indole moiety. It was further established that panobinostat and metabolites are excreted by both kidney and liver.[23]

Liquid chromatography combined with electrospray ionization mass spectrometry was employed for the investigation of chronic doxorubin cardiotoxicity in rats. The measurements indicated that doxorubicin influences the cardiac cytochrom P450-mediated arachidonic acid metabolism.[24]

Ultra performance liquid chromatography (UPLC) followed with electrospray ionization quadrupole time of flight mass spectrometry was applied for the investigation of the metabolites of the antitumor drug noscapine. The

method detected some new primary phase metabolites such as an N-demethylathed product, two hydroxylated derivatives, one bis-demethylated derivative. Several novel glucuronides have also been detected.[25] A high performance liquid chromatographic method coupled with electrospray mass spectrometry (LC-ESMS) was developed for the investigation of the structural and functional diversity in DNA-targeted hybrid anticancer drugs. It was stated that the new method can be successfully employed for the detemination of structure-activity relationships and for the identification of target compounds.[26]

The structure of the new anticancer agent EAPB0203 and its metabolites were investigated in detail. Measurements were carried out using liquid chromatography-mass spectrometry and LC/MS/MS. The structures of the metabolites were identified by one and two dimensional H-NMR.[27] A new immunoassay method was developed for the determination of 5-fluorouracil (5-FU) in patients with gastric cancer. The results were compared with those obtained with the application of GC-MS. It was established that the results of the immunoassay method and GC/MS were comparable and can be employed simultaneously for the determination of 5-FU in the plasma of patients with gastric cancer. It was further established that the immunossay method is simpler and easier to carried out than the traditional GC/MS procedure.[28]

Chiral chromatography combined with other physicochemical analytical procedures has been successfully applied for the investigation of the chemistry and pharmacology of Imexon and related cyanoaziridines. The measurements indicated that the activity of these new compounds was relatively low compared with the activity of the original compounds.[29]

The interaction between of RAPTA-T, a new ruthenium-based anticancer agent and human ovarian cells was investigated in detail. SEC has been employed for the determination of the distribution profile of metallodrug within the cancer cells. Multidimensional protein identification technology was employed for the elucidation of the cellular response mechanism. It was established that the investigation methods employed may help the elucidation of the interaction between the cancer cells and the anticancer drug.[30] A combining data acquisition method followed with multi-period ion scan and high resolution characteristics extracted ion chromatograms were employed for in-vivo drug metabolites screening and identification of the metabolites of the potential antitumor agent 3,6,7-trimethyloxyphenantroindolizine (CAT) in rat urine. It was established that the method is suitable for the detection of 21 metabolites and for the identification or 9 metabolites in rat urine. Furthermore the method allowed the differentiation between N-oxidized and hydroxylated metabolites.[31] A new type of folic acid conjugated amphiphilic 4-arm star-shaped PLGA-PEG-NH_2 were synthesized and tested as nanocarrier for doxorubicin. The characteristics of the preparation were assessed by H-1 NMR, dynamic light scattering and GPC. It was established that the new nanocarrier can be successfully applied for the deliver of doxorubicine.[32] The composition of the ethanolic extract of the chemopreventive Taiwanese mushroom *Antrodia camphorata* was investigated by HPLC. The measurements indicated that the triterpenoid rich fraction contained antileukemia components such as anticin, dehydroeburicoic acid, and zhankuic acid. It was found that the bioactive components decrease tumor weight and size without decreasing body weight. The preparation was proposed for the treatment of leukaemia.[33]

Capillary elctrophoresis (CE) combined with laser-induced fluoescence detection (LIF) has been employed for the study of the interaction of the antitumor agent ellipticine with DNA. The background electrolyte contained 20% dimethyl sulfoxide and 50 mM sodium acetate (pH 4.5). It was established that the peak of ellipticine decreased in the presence of DNA indicating the interaction of ellipticine with DNA.[34]

A HPLC/MS method was developed for the determination of CM1, the active agent derived from the prodrug CA1P, in human plasma and urine, and of CAIP and three glucuronides CA1G1, CA1G2, and CA1DG in human urine were analysed. Plasma CA1was preconcentrated by solid phase extraction (SPE). Urine samples were investigated without extraction. The intra-day and inter-day accuracy and precision were lower then 15%. Mean recovery of CA1 from plasma was 101% and 97% from urine. Mean urine recovery was of CA1P was 98%, CA1G1 96%, CA1G2 93% and CM1DG 93%.[35] A new GnRH-based multifunctional drug delivery system containing daunorubicin and methotrexate was developed and the in vitro stability, degradation in human serum, and in the presence of rat liver homogenate was investigated by LC/MS. It was concluded from the results that multifunctional bioconjugate resulted in higher in vitro cytostatic effect than the monofunctional bioconjugate contain either methotrexate or daunorubicin.[36]

Fluorinated, and pegylated polyaspartamide copolymers were synthesized to increase the solubility and efficacy of flutamide. The characteristics of copolymers were determined by SEC, pyrene colorimetric assay, light scattering analysis and scanning electron microscopy. The results indicated that the inclusion of flutamide in polymer matrices markedly modifyes the efficacy of the complexed bioactive compound.[37] The characteristics of the newly synthesized anticancer agent, 7-(4-fluorobenzylamino)-1,3,-4,8-tetrahydropyrrolo[4,3,2-d,e]quinolin-8(1H)-one (FBA-TPQ) were investigated employing various physicochemical and biophysical investigation methods such as rapid resolution liquid chromatography, determination of FBA-TPQ in plasma and tissue samples, stability in plasma, plasma protein binding, metabolism by S9 enzymes, plasma pharmacokinetics, and tissue distribution. It was concluded from the data that FBA-TPQ is a potential therapeutic agent for pancreatic cancer.[38]

The pharmakokinetics and antitumor activity of XMT-1001, a new polymeric topoisomerase I inhibitor was investigated in mice bearing HT-29 human colon carcinoma xenografts. LS/MS method was employed for the determination of the chromatographic profile of the drug and its conjugate release products. It was established that the efficacy of drug was markedly modifyed by formulation.[39]

An LC-MS/MS technology was developed and applied for the simultaneous determination of highly hydrophobic pyrimidine anticancer agents in human plasma and the efficacy of three derivatization reagents were compared. The anticancer agents included in the experiments were tegafur, 5-fluorouracil and gimeracil. Derivatization agents employed were p-bromophenacyl bromide, dansyl chloride

and diazomethane. The experiments revealed that the p-bromophenacyl was the best derivatization agent. The method was employed for the pharmakinetic study of tegafur, 5-fluorouracil and gimeracil in cancer patients.[40] The purification and characterization of lunasin, a peptide isolated from soy bean has been recently reported. The interest in lunasin is due to its marked anticancer and anti inflammatory actvity. A combined method was developed for the purification of lunasin from soybean. The method includes anion exchange chromatography, ultrafiltration and reversed-phase chromatography. It was stated that the procedure facilitates the development of lunasin as a potentional nutraceutical of therapeautic anticancer agent.[41]

The safety and pharmacokinetics of α-tocopheryloxy acetic acid (α-TEA) was investigated using LC/MS technologies. The measurements were motivated by the antitumor activity of α-TEA. According to the results α-TEA showed no toxic effect, the half life of orally administered alpha-TEA was 52 h. No clinical signs of toxicity was detected. No significant sex specific differences were established.[42]

A novel LC-MS/MS procedure was developed for the quantification of E7080 (lenvatinib) and metabolites in various human biological matrices such as human plasma, whole blood, urine and faeces. Plasma, urine, and faeces samples were extracted with acetonitrile. Analyses were carried out on an octadecyl-silica column (50 mm x 2.1 mm). Analytes were separated using gradient elution consisting of water-acetonitrile mixtures and detected with API300 triple quadrupole mass spectrometer with turbo ion spray interface operating in positive ion mode. Cailbration curves were in each case linear, accuracy varied between ±20%. It was found that the method can be successfully employed for the analysis of E7080 and its metabolites in various human biological matrices.[43]

A liquid chromatography-tandem mass spectrometry (LC-MS/MS) analytical procedure was applied for the separation and quantitative determination of felotaxel (SHR110008) in tumor-bearing mice. Samples (plasma, urine, faeces, brain heart, liver, lung) were extracted with ethyl acetate. Calibration curves were linear (r^2=0.995). The accuracy and precision varied between 86.1–107.2 % and 1.1-9.2%. Recoveries ranged from 73.9 to 96.1 %.[44]

A HPLC method was employed for the analysis of doxorubicin (Dox) loaded poly(alkyl cyanoacrylate, PACA) nanoparticles. The measurements indicated that Dox and its main metabolites doxorubicinol and doxorubicinon are well separated from the target compound. It was further established that recovery varied between 71 and 78%. The between –day and within-day precision of the method varied from 97.1 to 102.9% and from 97.3 to 1.02 %.[45] Another LC-MS method was developed for the investigation of pharmacokinetics of the anticancer drug CYC-116 in rat plasma. Analytes were separated on an octadecysilica column (150 x 04.6 mm, particle size, 5 µm) using isocratic separation mode. The flow rate was 0.8 mL min^{-1}. Isocratic mobile phase consisted of acetonitrile-water-formic acid: 23.5; 76.5; 0.1 v/v/v). Analytes were extracted by liquid-liquid extraction using ethyl acetate. MS analysis was carried out employing a quadruple mass spectrometer. The calibration curve was linear in the concentration range investigated. The intra- and interday precisions were less then 11.8–6.6 %. It was stated that the method can be successfully applied for the study of the pharmacokinetik of CYC-116 in rats after oral administration.[46]

A LC/MS/MS procedure was developed for the simultaneous determination of eleven thiopurine nucleotides (mono-, di- and triphosphates of thioguanosine, methylthioinosine, methylthioguanosine, and thioinosine). Target compounds show marked anticancer and immunosuppressive activity. It was established that the method facilitate the understanding of the thiopurine metabolism in one run.[47]

The application of thermochemotherapy mediated by novel solar-planet structured magnetic nanocomposite for glioma treatment has been reported. The efficacy of the encapsulation of docetaxel and drug release was followed by HPLC. It was concluded from the results that this novel method can be successfully employed for the comprehensive treatment of glioma.[48]

The stability of 5-fluorouracyl under different environmental conditions was investigated by using HPLC and infrared spectroscopy (IR). Analytes were separated on an octadecylsilica column using 40 mM KH_2PO_4 as mobile phase. Target compounds were detected at 260 nm. Significant linear correlation was found between the concentration of detector response and the amount of analytes in the samples (r^2=0.9995). The R. S. D. values for intra-day and inter-day were <0.2% and <1%, respectively. The measurements indicated that the analyte is sensitive to oxidative conditions and stable when exposed to UV irradiation.[49]

Another LC-MS/MS technique was developed and successfully employed for the simultaneous determination of doxorubicin and its metabolite in cultured human leukemia cells. Samples were enriched using solid phase extraction, the validated calibration ranges were 5.00 – 1000 mg mL^{-1}. The data indicated that the method is precise and can be applied for the simultaneous determination of the drug and its metabolite.[50]

Niosomal formulations were developed, characterized and applied as potential brain targeted system. Light scattering and transmission electron microscopy were applied for the characterization of vesicles. SEC or dialysis was applied for the investigation of the physicochemical parameters of vesicles. It was concluded from the results that the method in suitable to improve the doxorubicin brain delivery.[51] A novel (NAG)-PEG-doxorubicin targeted conjugates were designed, developed, synthesized for its capacity as anticancer delivery agent. The product was characterized by 1H.NMR, UV spectroscopy and HPLC. It was concluded from the results that the new products can be applied in targeted anticancer therapy.[52] The in vivo performance and in vitro cytotoxicity of 10-hydroxycamptothecin (10-HCPT) was investigated in detail. The effect of 10-HCPT nanosuspernion and 10-HCPT solution was compared and critically evaluated. HPLC-FD was employed for the analysis of serum samples and tissue homogenizates. It was further found that 10-HCPT increases the cytotoxycity of the original anticancer agent. Increase the anti-tumor activity of poorly soluble bioactive compound.[53] The solubility of paclitaxel (PTX) was improved by using various coprecipitation processess including poly (L-lactic

acid, PLLA). The components of the formulation were mixed and coprecipitated by a supercritical antisolvent (SAS) process using dichloromethane (DCM) and mixtures of DCM and ethanol, or DCM and dimethyl sulfoxide (DMSO). The end product was characterized by X-ray diffraction (XRD), scanning electron microscopy (SEM), laser diffraction, particle size analysis, and HPLC. The experimental conditions for the optimal system were: DCM/EtOH 50:50 %, 35 ^{0}C, 10-12 MPa, PLLA concentration, 5 g L^{-1}; solution flow rate 0.5 ml min^{-1}.[54]

The anticancer applications of mesoporous materials functionalized with the natural betulinic acid (BA) has been investigated in detail. Dehydrated CMC-41 was functionalized with 3-chloropropyltriethoxysilane (CPTS) and 3-aminopropyltriethoxysilane (APTS). The end product was characterized by powder X-ray diffraction, X-ray fluorescence, nitrogen gas sorption, multinuclear MAS NMR spectroscopy, thermogravimetry, UV spectroscopy, IR, SEM, and HPLC.[55]

The characteristic of methoxy stilbenes as potent, specific, untransported and cytotoxic inhibitory of breast cancer resistance protein has been studied in detail. It was established by HPLC and mass spectrometry titration that methoxystilbenes are not transported. It was further suggested that the methoxy derivatives of stilbene can be applied to influence drug-efflux activity.[56] The light-mediated release of the anticancer drug methotrexate was investigated employing three different analytical methods such as UV/vis spectrometry, ^{1}H NMR spectroscopy and HPLC. The measurements indicated that the drug is released by the photochemical mechanism in an actively controled manner. It was further found that the release of methotrexate depended on the linker design, light wavelenght, exposure time, and the pH of the medium.[57]

An octadecyl-silica column was applied for the separation and quantitative determination of some new benzimidazole derivatives with marked cytotoxic activity. The stability of the target compounds was investigated in aqueous solution of 0.2% dimethyl sulfoxide. The mobile phase of the HPLC measurements consisted of acetate buffer (pH 4.5):acetonitrile. The flow rate was 1.0 mL min^{-1}. The correlation coefficients were over r^2 = 0.9995. The new method was proposed for the HPLC analysis and stability test of these novel benzimidazole derivatives.[58]

During the study of the pharmacokinetics of the amino-pairing peptide EAK16-II (EAK) it was established that EAK is suitable for the stabilization of EAK-EPT (ellipticine) complexes in vivo. The characteristics of the EAK-EPT complexes were investigated by transmission electron microscopy TEM), HPLC and zero potential measurements. EPT was extracted from rat plasma with dexamethasone sodium phosphate. The measurements found significant differences between EPT and EAK-EPT complexes indicating that EAK can be applied as a carrier to increase the biovailability of EPT.[59]

The design and synthesis of photoactivatable metallodrugs has been recently published. The compounds included in the experiments were: inert (Ru(II) half sandwich compounds, [Ru([9]aneS(3)(bpy)(py)][PF_6] ([9]aneS(3)=1,4,7-trithia-cyclononane, bpy=2,2'-bipyridine, py=pyridine), [Ru([9]aneS(3))(en)(py)][PF_6] (en=1,2-diaminoethane), and [Ru([9]aneN(3))(en)(dmso-S)][PF_6], [9]aneN(3)=1,4,7-triazacyclononane), is reported along with the X-ray crystal structure of [Ru([9]aneS(3)(bpy)(py)][PF_6]. It was established that these complexes are promising for further investigation as potential photochemotheropeutic agents.[60]

A new potential PET (positron emissin tomography) agent was synthesized for imaging steroid sulfatase (STS) in cancers. Solid phase extraction combined with HPLC was employed for the purification the target tracer.[61] The murine pharmakokinetics, pharmacodynamics and metabolism of (3-(1H-indol-2-yl-phenyl)(1H-indol-2yl-methanone) (in-dole-15). The concentration of indole-15 was determined by HPLC and LC/MS/MS. The investigation of metabolites indicated that the original compound undergoes extensive oxidative metabolism with following surfation. It has been proposed that indole-15 can be employed in clinical trials.[62] A specific and sensitive enzyme-linked immunosorbent assay (ELISA) was developed for the analysis of Vindesine (VDS) and the results were compared with those obtained by HPLC. Anti-VDS antibody was obtained by immunizing rabits with VDS conjugated with bovine serum albumin using N-[β-4-(diazophenyl)ethyl]maleinimide. The enzyme marker was produced by coupling VDS with horse radish peroxidase using N-4-diazophenylmaleinimide. The method was highly specific the cross reactivity was 0.18% for vincristine and 0.11 % for vinblastine. It has been stated that the method can be successfully employed for the pharmacokinetics studies of (VDS).[63] The mass balance, excretion and metabolism of the small molecule flavonoid tumour vascular disrupting agent ASA404 was investigated using various chemical, physicochemical methods such as HPLC, liquid scintillation counting, mass spectrometry, glusulase. The mean recoveries were: urine, 59.9%; faeces, 33.3%. It was found that the method is suitable for the detection and analysis of two new metabolites did not detected before.[64]

A new HPLC method was developed for the study of new anticancer agents in intravenous solutions. Compounds included in the exteriments were the derivatives of daunorubicin containing various amidine group such as piperidine (DD-1), morpholine (DD-2, pyrrolidine (DD-3), or hexahydroazepine (DD-4) moiety. It was found that the environmental conditions exert marked effect on the stability of the preparation.[65] A TAT peptide fragment (YGRKKRQRRR) was conjugated to a platinum(IV) analogue of oxiplatin as a vehicle for membrane penetration. The mono- and difunctionalized conjugates were separated by preparative HPLC and characterized by analytical HPLC, ESI-MS, and H-1 NMR spectroscopy. The results indicated that the biological activity of conjugates was higher then that of the untargeted analogues.[66] The influence of of miRNAS a regulator in some biological processes such as development, cellular differentiation, and carcinogenesis was investigated in detail. The promising anticancer drug 3,6-dihydroxyflavone (3,6-DHF) was selected as model compound. HPLC was employed for the detection of the bioavailability of 3,6-DHF. Cell apoptosis was determined by flow cytometry or terminal deoxynucleotidyl transferase UTP nick end-labeling assay. The investigations indicated that the oral administration of 3,6-DHF suppressed the breast carcinogenesis inducated by 1-methyl-1-nitrosourea (MNU) in rats. It was further found that 3,6-DHF is a potent natural chemopreventive agent, and miR-34a and miR-21 play a considerable role in MNU-induced breast

carcinogenesis.[67] Long conjugated 2-nitrobenzyl-derivative caged anticancer prodrug with visible light regulated release was synthesized. Styryl conjugated 2-nitrobenzyl derivatives were introduced in the molecules as phototrigger to regulate the release of the anticancer drug chrorambucil. UV absorption, FT-IR and HPLC technologies were applied for the study of the regulated release of the parent molecule.[68] The efficacy of a non-hypercalcemic vitamin-D-2 derived anti cancer agent (MT19c) was investigated. The investigation indicated that the new compound makes possible of the future potential application of this compounds as anticarcinogen agent.[69]

Reversed phase thin layer chromatography has also found applications in the chromatographic analysis of bioactive compounds. Stationary phases consisted of octadecyl silica and silica surface modified with cyano groups. Methanol-water and acetonitrile-water mixtures served as stationary phases. Significant correlations were found between the lipophilicity determined with RP-TLC and calculated with principal component analysis. The chromatographic parameters of bioactive compounds included in the experiment were correlated with the biological activity of the analytes (quantitative structure-retention relationship and quantitative structure-activity relatiohship). The measurements indicated that the there is a significan correlation between the lipophilicity and molecular descriptors of phytol derivatives. It was assumed that the chromatographic behaviour of phytol derivatives may influence their penetration and partitioning over biomembranes.[70]

Abbreviations

APTS	3-aminopropyltriethoxysilane
CA	Capillary electrophoresis
CPTS	3-chloropropyltriethoxysilane
DCM	dichloromethane
DHF	3,6-dihydroxyflavone
DMSO	dimethyl sulfoxide
FTIR	Fourier transformed infrared spectroscopy
GPC	gel permeation chromatography
HPLC	high performance liquid chromatography
MNU	1-methly-1-nitrosourea
IR	infrared spectroscopy
PET	positron emision tomography
PLLA	poly-L-lactic acid
PTX	paclitaxel
LIF	Laser-induced fluorescence detection
RP-HPLC LC-MS/MS	reversed-phase high performance liquid chromatography-tandem mass spectrometry
RSD	relative standard deviation
SEM	scanning electron microscopy
SEC	size exclusion chromatography
SPE	solid phase extraction
UPLC	ultra performance liquid chromatography
XRD	X-ray diffraction

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