Current HPLC Methods for Determination of Medicaments in Formulations and Biological Samples

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ABSTRACT

The performance of high-Performance liquid chromatography (HPLC) instruments has been remarkably progressed. As a result, an HPLC method plays a conspicuous role in analysis of medicaments in formulations and biological samples. The varied detection methods used for HPLC such as ultra violet (UV), mass spectrometry (MS), fluorescence (FL) and chemiluminescence (CL) detections in addition to electrochemical detection (ECD) with suitable pretreatment or labeling were chosen in response to the purpose of analysis. Analysis of medicaments in formulations was mainly performed by UV detection, meanwhile EC, MS, FL and CL detections with high sensitivity were used for analysis of medicaments in biological samples. The sensitivity ranging from microgram to picogram level could be achieved. In this review, current HPLC methods for determination of medicaments in formulations and biological samples were described. Furthermore, their advanced applications for chiral analysis and pharmacokinetic drug-drug interaction evaluation of medicaments were presented.

Keywords: HPLC, Medicaments, Formulations and Biological Samples.

INTRODUCTION

Analysis of medicaments in formulations or biological fluids is required to promote for rational use of medicament. Any useful information for quality control, pharmacokinetics, pharmacodynamics, pharmacology and toxicology of medicament can not be obtained without it. Especially, therapeutic drug monitoring based on determination of medicaments is one of the requisite factors for performing appropriate treatment by some medicaments(1).

Varied methods have been used to analyze medicaments. In clinical practice, immunoassays which offer appropriate sensitivity for the detection of medicaments in biological specimens are utilized such as fluorescence (FL) polarization immunoassay(2), chemiluminescence (CL) immunoassay(3) and enzyme-linked immunosorbent assay(4). Exclusive kits and automated instruments for each medicament have been developed and widely spread. However, the cross reaction sometimes makes overestimation for the determination of each component in the samples(5).

On the other hand, a separation technique is required in the cases as follows:

1. Simultaneous determination of coadministrated medicaments such as anticonvulsants.
2. Simultaneous determination of parent compound

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and active metabolites.

3. Analysis of racemic compounds.

Among separation techniques employed for medicament analysis such as thin-layer chromatography[6], gas chromatography (GC), high-performance liquid chromatography (HPLC)[7], capillary electrophoresis[8] and capillary electrochromatography[9], GC and HPLC have proven to be the most popular, because these can offer better sensitivity, selectivity and applicability. Especially, HPLC shows excellent capability for the analysis of aqueous samples. Most medicaments as well as endogenous components in biological samples are commonly non-volatile polar compounds, and thus HPLC is more suitable than GC for their analysis.

One of the major advantages of HPLC for analysis of medicaments is the improvement of stationary phase. The separation of medicaments with more than several ten thousands theoretical plates/m can be performed. Though a conventional reversed-phase ODS (C18) column is the most frequently used for this purpose, other kinds of stationary phases such as C4[10], C8[11-16], C30[17] and NH_2[18-20] columns are also chosen according to a characteristic of analyte. A normal-phase silica column is also used for analysis of medicaments[21] or those with labeling[22] due to their hydrophobic properties. Recently, a monolithic column has attracted attention as an alternative option for HPLC. Since monolithic support plays as continuous homogenous phases, users can perform chromatography with much lower pressure than that of conventional packed column. Therefore, much faster separations at high flow rates can be achieved with high efficiency and reduction of running time. Separations of β-lactamic antibiotics (*e.g.,* amoxicillin, ampicillin and cephalexin)[23], and sotalol[24] were performed with a monolithic column. Furthermore, a specific column for chiral separation of medicaments can be available[21, 25-31].

Another advantage of HPLC is a variety of detection methods including ultra violet (UV), mass spectrometry (MS), FL and CL detections in addition to electrochemical detection (ECD). An operator can select the suitable detection method in the aims of analysis, *e.g.,* sensitive, selective, rapid, simple or inexpensive determinations.

The aim of this review is to overview current HPLC methods for determination of medicaments in formulations and biological samples. The varied detection methods of HPLC such as UV, MS, FL, CL and ECD will be mentioned. In each following section, representative interesting results including our recent publications were presented. Furthermore, their advanced applications for chiral analysis or pharmacokinetic drug-drug interaction evaluation of medicaments will be mentioned.

HPLC Methods for Determination of Medicaments in Formulations

Medicaments in formulations

Numerous HPLC methods for determination of medicaments in formulations have been reported. These methods have been applied to determine medicaments in various formulations such as tablet, capsule[23,32], dermatological formulation[33], multi-component syrup[34] and aerosol[10]. In general, since formulations contain relatively large amounts of medicaments ranging from sub-microgram to several hundred milligram/formulation, highly sensitive determination is not always necessary. On the other hand, a strict-validated analytical method is required. The objective of validation of an analytical procedure is to demonstrate whether it is adequate or not for its intended purpose. A number of parameters must be investigated in order to validate the analytical methods, e.g., precision, accuracy, specificity, limits of quantification (LOQ) and detection (LOD), linearity and robustness defined by ICH[35]. Precision is measured as repeatability, intermediate precision and reproducibility. The repeatability (intra-day) and intermediate (inter-day) precision of the method are demonstrated by analyzing the sample reference standard solution of known concentration during 1 day and each of several days under the same conditions. Reproducibility refers to use of the analytical procedure in different laboratories. Accuracy is

determined by analyzing a sample of known concentration of standard spiked in sample and comparing the measured value with the true value. Specificity of method is determined by a placebo analysis. Placeboes containing all additives except ingredient are prepared for this study. They are treated in the same manner as the normal samples. To compare the chromatogram of sample with that of placebo, the specificity of the method could be confirmed. The LOQ and LOD are calculated as correspondence concentrations with a signal-to-noise (S/N) ratio of 3 and 10, respectively. In addition to this, the LOD (or LOQ) is expressed in equation (1),

$$\text{LOD (or LOQ)} = 3.3 \text{ (or 10) } \sigma/s \qquad (1)$$

where σ is the standard deviation of the response and s is the slope of the calibration curve, and defined as a five (or three) times of standard deviation of the appropriate blank response. The LOQ was defined as the concentration which gives less than 20 % of RSD and/or less than ±20 % of accuracy. Linearity should be evaluated by an appropriate statistical method such as least squares method. For establishment of linearity, a minimum of 5 concentrations is recommended by ICH. The robustness of the method is a measure of its capacity to remain unaffected by small, but deliberate, variations in the method parameters such as pH value of mobile phase, and provides an indication of its reliability during normal usage. Heyden *et al*. reviewed for robustness in method validation[36].

A part of current HPLC methods for determination of medicaments in formulations is summarized in Table 1. Since matrix of formulation is not so complicated, time-consuming pretreatment and a complicated elution system for separation of medicaments are not required. Almost medicaments are extracted by ultrasonication. The most methods listed in Table 1 utilized UV detection. An HPLC-UV method is simple and practical for determination of a medicament having a chromophore with absorption bands in the UV or visible region.

Medicaments in biological samples

The varied matrices such as urine, blood (whole blood, serum and plasma), tissue homogenate and dialysate were used for biological analysis of medicaments. Commonly pretreatment of biological samples is required before injection into an HPLC system. The simplest pretreatment is deproteinization with denaturants such as organic solvents or acids. After deproteinization, the mixture is centrifuged and the resultant supernatant is subjected to analysis. A deproteinization method is advantageous as it is convenient and more economical compared to a liquid-liquid extraction and solid-phase extraction (SPE) methods.

Liquid-liquid extraction is another alternative for sample clean-up. Water-immiscible organic solvents are used for extraction of analytes from biological fluids. SPE has become more popular for analyses of medicaments in biological fluids due to the excellent figures such as selective retention of the analytes, reduction of sample size and solvent requirements, high throughput and the possibility of automation. Recently, liquid-phase (LPME) and solid-phase microextraction (SPME) are known as new and effective sample preparation techniques. In LPME, medicaments can be extracted from aqueous biological samples, through a thin layer of organic solvent immobilized within pores of the wall of porous hollow fiber[48, 49]. For SPME, fibers and capillary tubes coated with an appropriate stationary phase are usually used. Moreover, microextraction in a packed syringe and stir-bar-sorptive extraction using coated magnetic stir bar have been developed[50]. In both techniques, microliter level of solvent was required, and thus the solvent consumption in pretreatment could be minimized.

Ultrafiltration, a straightforward sample preparation method, is also utilized as a convenient and rapid pretreatment of biological fluids to avoid tedious extraction and evaporation techniques. Aliquots of plasma or serum sample are simply filtered by centrifugation with a micron filter, and the resultant is injected onto an HPLC system.

A direct injection method is a preferred technique for the analysis of biological fluids concerning time- and

cost-consumption, analyte loss and simplicity. Direct injection is generally combined with column switching. The column switching technique allows the adsorption of analyte on a pre-column and removal of the interfering components such as protein, followed by back-flushing and transfer of the analyte to an analytical column by switching the direction of the effluents[51].

Microdialysis is often utilized as a sampling tool to monitor concentrations of free-form drugs or neurotransmitters, and is also applied to neuropharmacological and pharmacokinetic studies. A microdialysis probe consists of a small semipermeable membrane, on which analytes are recovered by passive diffusion (Fig. 1). As the advantages of microdialysis, long-term sampling is possible with minimal damage for organs or tissues and the clean-up procedure is not ordinarily required[52]. Many application studies on pharmacokinetics and drug-drug interactions using microdialysis have been reported[17, 53, 54].

A highly sensitive analytical method determining less than sub-nanogram level is generally required for analyses of medicaments in biological samples. In pharmacokinetic studies, low concentration levels of medicaments in samples should be determined for several hours after administration. Sensitivity of the analytical method is required according to the following conditions;

・Dose of medicaments.
・Stability of medicament in living body.
・Amount of matrix obtained.

For this purpose, HPLC-MS (or -MS/MS), -FL, -CL or -ECD were mainly used (Table 2). Characteristics of these methods will be mentioned in the next paragraph. In some cases, a labeling reaction was combined with these methods as the occasional demands. Labeling can serve improve the sensitivity, selectivity and chromatographic behaviors.

HPLC-UV detection

The advantages of HPLC-UV detection are wide adaptablity and simplicity using a relatively inexpensive instrument. The sensitivity of the analysis of medicaments by UV detection depends on their absorptivity, and is generally inferior to those of MS, FL or ECD. However, an HPLC-UV method is precise and frequently used for quality control of medicaments in formulations. Because medicaments contained in formulations are relatively in large amounts, precise identification and quantification of the active components and the impurities are important for the safety and efficacy of formulations. The impurities and potential degradation products can be present in bulk and formulations and change the chemical, pharmacological and toxicological properties. Rao *et al.* reported a rapid and simple HPLC-UV method for paracetamol[38]. Paracetamol, nine impurities and one degradation product in the formulation were successfully determined within 50 min. Stability-indicating assays for SK3530[37], ofloxacin[39], donepezil (DP) hydrochloride[44] and phenylpropanolamine (PPA)[82] in bulk and formulations using HPLC-UV detection were also reported. In our previous report, PPA, caffeine and chlorpheniramine in commercially available over-the-counter preparation were well separated (Fig. 2) and simultaneously quantified with the limit of detection of sub-millimolar levels[45].

On the other hand, since the performance of UV detector has been recently improved, the determination of medicaments in biological samples was also achieved by an HPLC-UV method. Levomepromazine, clozapine and their main metabolites in human plasma[11], aspirin in human serum[59], docetaxel and paclitaxel in plasma[83], DP in plasma[27, 84], fluoroquinolones such as enoxacin, ofloxacin and norfloxacin in chicken blood[15], non-steroidal anti-inflammatory drugs, ketoprofen, meloxicam[28], flurbiprofen[29], ibuprofen and dicrofenac sodium[60] and triazolam[53] in plasma were determined. An HPLC-UV detection was also applied to pharmacokinetic studies of tenatoprazole[55], ferulate sodium[56], clopidogrel[14] and paclitaxel[58,85,86]. However, relatively large amounts of blood sample (200-1000μl) were consumed for determination of medicaments.

HPLC-ECD detection

The ECD detection is accepted as a sensitive and selective technique for the determination of electroactive substances and has been used for quality control of medicaments. Levodopa methyl ester[25] and tanshinone IIA[87] in formulations containing electroactive functional groups such as phenol were detected with LOD of a few nanogram per milliliter. Nakao *et al.* reported an HPLC-ECD detection method for quality control of positron emission tomography (PET) radiopharmaceuticals[12]. In 19 PET pharmaceuticals studied involving methionine, dopa, methylspiperone and verapamil, these compounds and their corresponding precursors used in the synthesis of the radiopharmaceuticals were quantified. This method could be applied to the analysis of [^{11}C] MP4A, a useful PET radiopharmaceutical having no available UV absorbance for measuring acetylcholinesterase activity in the brain.

Moreover, determination of medicaments in biological samples, e.g., urine, plasma, serum and tissue, by HPLC-ECD was demonstrated with suitable pretreatment of the sample such as liquid-liquid extraction or SPE[30,61-64,87,88]. However, the ECD is hardly compatible with gradient elution. This fact is the main disadvantage for ECD, and generally limits its use to the sole separation of a couple of medicaments, *e.g.,* metoclopramide, imipramine, diclofenac and hydrochlorothiazide, in a single run[87]. Therefore, the analysis of single medicament by ECD has been extensively performed for clenbuterol[62], 5-hydroxyoxindole[63] and vitamin K[64]. However, using a new generation coulometric detector, simultaneous determination of ten commercially available macrolide antibiotics such as erythromycin, dirithromycin, tylosin, tilmicosin, spiramycin, josamycin, kitasamycin, rosamicin, roxithromycin and oleandomycin in human urine was achieved with LOD of 2.5 ng on column[61].

HPLC-MS (/MS) detection

Measurement of small amounts of medicaments and pharmacodynaminc biomakers in biological samples provides the opportunity for a better understanding of drug efficacy and toxicity. HPLC-MS is a powerful analytical tool that can facilitate the measurement of medicaments and biomarkers[89]. Compared with GC-MS, HPLC-MS is well-suited for sensitive quantification of polar and non-volatile medicaments and has become a widely applied technique in recent years. MS detection provides both qualititative and quantitative information for each analyte. HPLC-UV, -FL and -ECD rely on the retention time of the analytes, whereas MS can identify the analytes even in the presence of co-eluted impurities by using a single ion monitoring mode. The development of interfaces between HPLC and MS was very important to build up the valuable HPLC-MS system. An interface is devised to separate small amounts of analytes from large volumes of solvents and only can ionize analytes. Two ionization modes, an atmospheric pressure chemical ionization (APCI) and an electrospray ionization are mainly used for the interfacing of MS for the measurement of medicaments. The development of these ionization sources has enabled the evolution of this technique to the point where HPLC-MS is routinely used for pharmacokinetic studies[7]. Due to its high sensitivity and selectivity, an HPLC-MS method is mainly used for quantification of medicaments and biomarkers in biological samples. As shown in Table 2, a number of methods hase been established for determining of medicaments such as triptolide, wilforide A, triptonide[66], indapamide[67], cyclovirobuxine[68], gambogic acid[69] and dexamethasone[90].

Tandem MS (-MS/MS) is a tool in which two mass spectrometers are connected by a fragment chamber. Compared to HPLC-MS, more sensitive and selective determination could be accomplished with tandem MS/MS. MS/MS detection can distinguish the compounds which have the same intact mass and provide additional information enhancing the reliability of the method. Applying HPLC-MS/MS detection has led to the development of rapid and sensitive methods for analyses of many kinds of medicaments such as glucosamine[20], warfarin[31], cetirizine[16], rosiglitazone[91], neomycin and bacitracin[92] in body fluids. Vainchtein *et al.* developed an HPLC-MS/MS for

paclitaxel and its 3'-*p*- and 6-α-hydroxy metabolites in human plasma with a simple liquid-liquid extraction procedure[65]. Paclitaxel and its metabolites were rapidly separated within 9 minutes of run time and sub-nanogram per milliliter of substrates could be determined. Although MS/MS detection is very sensitive, it is too expensive to use for the routine measurements in the pre-clinical laboratory.

HPLC-FL detection

An HPLC-FL detection method has been extensively employed for the determination of trace amounts of compounds and favorably comparable to UV detection in regard to sensitivity and selectivity, because FL detection counts the increase in photon number occured, while UV detection measures the ratio of transmitted light that passes out through the sample solution and the original irradiation light. Additionally, for FL detection, two wavelengths for excitation and emission should be selectively used; this is the major reason why FL detection is more selective than UV detection. Furthermore, its instruments are inexpensive compared with MS (or MS/MS) detection.

Medicaments with their intrinsic FL can be detected by HPLC-FL without pretreatment such as labeling. For instance, recombinant human erythropoietin in pharmaceutical products was determined by anion-exchange chromatography[93]. Protein binding of naproxen based on measurement of its intrinsic FL was studied[48]. In biological samples (mainly plasma), analyses of nabumetone[73] and its metabolites[72], camptothecin[74] and doxazosin[75] were performed. In our previous report, by utilizing intrinsic FL of DP hydrochloride, rapid and sensitive determination of DP in human plasma, rat plasma and microdialysis was achieved with a short C30 column by an isocratic elution[19]. As shown in Fig. 3, DP was separated within 15 minutes and LOD of DP in human plasma, rat plasma and microdialysis ranged from 0.2-2.1 ng/ml (S/N=3). Moreover, pharmacokinetic studies of medicamensts such as MCC-555 (thiazolidineones)[94], sotalol[24] and atenolol[76] could be achieved by consuming small amounts of plasma because of their intense FL. However, in most cases, the intrinsic FL intensity of the medicaments is not strong and co-existing compounds in matrices interfere its determination.

If compounds do not have fluorescent properties described above, a labeling reaction is recommended in order to introduce such a characteristic in the molecule. A labeling reaction is commonly achieved by the selective reaction between the functional group of the analyte and that of the labeling reagent. Labeling serves to improve the sensitivity, selectivity and the chromatographic behavior. Many labeling reagents have been developed for each reactive functional group. Representative fluorescent labeling reagents are shown in Fig. 4. To date, several reviews describing the labeling reagents for HPLC have been published[95-98].

Amantadine[46], voglibose[18] and fluoxetine[26] in formulation were determined by an HPLC-FL method with FL labeling. Sensitive and reliable methods with 9-fluorenylmethyl chloroformate (FMOC, Fig. 3-c) for determination of vertilmicin[99] and alendronate sodium[78] were established. 4-(4,5-Diphenyl-1*H*-imidazol-2-yl) benzoyl chloride (DIB-Cl, Fig. 3-d) was one of the most powerful fluorescent labeling reagents and has been applied to determine the varied medicaments such as mazindol and its metabolite[76], PPA[54, 100] and atomoxetine[101]. Especially, the method for PPA showed applicability to the varied biological samples such as human plasma, rat brain and blood microdialysates. The proposed method showed high sensitivity with LOD of 0.14 ng/ml in plasma using only 100 µl of sample, and 2.4 and 2.9 ng/ml in brain and blood microdialysates, respectively, using 15 µl of microdialysates. This method could be useful for PPA monitoring in human plasma for clinical and forensic purposes.

However, FL determination of medicament having no functional group is difficult, because it can not be labeled with a typical fluorescent labeling reagent. Therefore, instead of mother medicament, its metabolite having a reactive functional group was used for analysis. 4-(4-Bromophenyl)-4-hydroxypiperadine, a bromperidol

metabolite was determined with 4-fluoro-7-nitro-2, 1, 3-benzoxadiazole (Fig. 3-a)[72]. Recently, 4-(4,5-diphenyl-1*H*-imidazol-2-yl) phenylboronic acid (Fig. 3-k), a novel fluorescent labeling reagent for aryl halides was developed[22]. This labeling based on Suzuki coupling reaction that is a palladium-catalyzed cross-coupling reaction of aryl halides with arylboronic acids. The proposed method was successfully applied to the quantification of haloperidol in human serum.

HPLC-CL detection

CL is an emission of light from luminescing species electronically excited by a chemical reaction of a precursor of those species. The CL reaction doesn't require an exciting light source. This permits an increase in the detector's sensitivity, the attainment of a large S/N ratio and an inexpensive instrumentation. Therefore, the CL detection method is of growing importance in pharmaceutical and biomedical analysis due to its low LOD, and a simple instrumentation[102]. The CL reactions including luminol, peroxyoxalate chemiluminescence (PO-CL) and electrogenerated chemiluminescence (ECL) such as tris(2,2'-bipyridyl)ruthenium(II) ($Ru(bpy)_3^{2+}$) and Mn(III) have been well-known. Several reviews for CL reactions have already been published[96, 102-104].

Among them, luminol is one of the most important CL reagents for the determination of medicament. It has known that oxacillin, an antibiotics prolong, can enhance the luminescence from luminol. Using this system, unbound oxacillin in human serum was determined with a simple ultrafiltration pretreatment[105].

In PO-CL system, oxalates or oxamides react with hydrogen peroxide to yield intermediate peroxides which produce light by energy transfer to a co-existing fluorophore[103]. Tsunoda *et al*. reported a sensitive determination method of catecholamines and 3-*O*-methyl metabolites by fluorescence labeling with ethylenediamine[106]. The proposed method was successfully applied to determine catecholamines in mouse plasma by on-line labeling. Additionally, penbutolol[107] labeled with 4-dimethylaminosulfonyl-7-(*N*-chloroformylmethyl-*N*-methyl)amino-2,1,3-benzoxadiazole and estradiol[108] labeled with dansyl chloride were also sensitively determined in plasma by HPLC-PO-CL. Recently, unique HPLC-PO-CL methods combined with UV irradiation were developed for the determination of medicaments, which were converted to hydrogen peroxide and fluorescent products. Selective determination of quinones[109] and atremisinin[79] were achieved. Typical chromatograms of artemisinin in human serum were shown in Fig. 5.

$Ru(bpy)_3^{2+}$ is electrochemically oxidized to $Ru(bpy)_3^{3+}$ on the electrode surface, then, CL detection occurs during oxidization of the various amines by $Ru(bpy)_3^{3+}$. β-blockers such as atenolol and metoprolol in human urine were determined by this method[80]. Another ECL where emission of light from oxidized indomethacin by Mn (III) electrogenerated on electrode surface was found[81]. By this method, sensitive determination of indomethacin in urine with a LOD of 8 ng/ml (S/N=3) was achieved without any pretreatment. Numerous applications using an HPLC-CL detection have been reported in proportion to the increase in chemiluminogenic and CL labeling reagents described above.

Advanced Application Research

Chiral analysis of medicaments by HPLC

Analysis of enantiomers is very important to provide data for pharmacokinetic studies when the enantiomers possess different pharmacological activities and/or are metabolized at a different ratio.

Chiral separation of medicaments can be achieved by a direct or indirect separation. A review described in details on chiral separation of medicaments has already been published[7]. In the direct separation, enantiomers or those labeled with an achiral reagent are separated with a chiral stationary phase. Recently, this direct separation is mainly used because the varied chiral stationary phases such as Pirkle-type, cellulose and amylase types can be selected for analytes. Enantiomers of DP[21,27] and flurbiprofen[29] were determined by an HPLC-UV method with Chiralcel OD and Chiralpak AD-RH columns, respectively. *R/S*-Warfarin

separated with a Chiralpak AD column was determined by HPLC-ACPI-MS/MS[31]. In the indirect separation, enantiomers labeled with a chiral reagent are separated on an achiral column (*e.g.*, ODS). The reports using an indirect separation are not so much, since quality control of the chiral reagents for labeling is very difficult. An HPLC-UV detection method for ketoprofen labeled with L-leucinamide was reported by Montoya *et al.*[28]. They evaluated the pharmacokinetic parameters of enantiomers in dog after an oral administration at the recommended dose. Recent chiral analysis methods of medicaments were summarized in Table 3.

Study on drug-drug interaction

Nowadays, multi-medicament use was increased in clinical practice, especially, in the treatment of elder patients. Therefore, the drug-drug interaction of medicaments, which are expected to be coadministrated in clinical practice, seems to be significant. Accumulation of this kind of knowledge may be helpful for rational use of medicament in practice. In this mean HPLC method is a powerful technique to study on drug-drug interaction.

A semi-micro column HPLC method for triazolam (TZ) in rat plasma and brain microdialysates was developed for drug-drug interaction study of triazolam with Itraconazole (ITZ)[53]. As a result, ITZ seriously interfered with pharmacokinetic parameters of TZ when single simultaneous administration of TZ with ITZ and single administration of TZ after daily pretreatment with ITZ were performed. Furthermore, established HPLC-FL detection for PPA labeled with DIB-Cl was applied to assess the possibility of pharmacokinetic interaction of PPA with caffeine and chlorpheniramine[100]. These medications are present in the same formula. In this study, coadministration of PPA with caffeine and chlorpheniramine showed significant alteration on pharmacokinetics of PPA in brain[54].

CONCLUSION

Nowadays, the analysis of medicaments plays important roles in the development of new medicaments, quality control of formulation and evaluation of therapeutic effect. In this paper, current HPLC methods for medicaments in formulations and biological samples are the subjects of interest. In the recent decade, performance including sensitivity, selectivity and rapidity of the methods has been rapidly progressed in proportionality to the progress of instruments, stationary phases and labeling reagents. Additional remarkable points of current methods are the reduction of consumption of the reagents and waste with consideration of our environment. In fact, the techniques for miniaturization of the system and shortening analytical time can contribute to achieve those goals. Hereafter, HPLC will keep occupying an essential position to carry on the analysis of medicaments.

Table 1. HPLC methods for medicaments in formulations.

Medicament	Formulation	Pretreatment	HPLC conditions	Notes	Ref
[UV detection]					
Oristat	Capsule	Ultrasonication	Nova-pack C18, 0.1% Phospholic acid/CH_3CN (=10:90, v/v), 205 nm	LOD: 3 µg/ml (S/N=3)	32
Tretioin, isotretinoin	Dermatological formulation	Ultrasonication	Nucleosil C18, 0.01% TFA/ CH_3CN (=15:85, v/v), 342 nm	LOD: 0.06 µg/ml.	33
a) Guaiphenesine (GA), salbutamol (SL), methylparaben (MP), propylparaben (PP); b) acephylline piperazine (AC), bromhexine (BX), methylparaben (MP), PP	Multicomponent syrup		Shim-pack RP18, a) 50 mM H_2KPO_4 (pH 4.3)/CH_3CN (=60:40, v/v), 243 nm, b) 50 mM H_2KPO_4 (pH 3) /CH_3CN (=50:50, v/v), 245 nm	LOD: a) 17.1 ng/ml (GA), 17.0 ng/ml (SL), 17.6 ng/ml (MP), 16 ng/ml (PP); b) 17.2 ng/ml (AC), 17.9 ng/ml (BX); 17.9 ng/ml (MP), 15.2 ng/ml (PP).	34
Dexpanthenol	Aerosol, gel	Ultrasonication	Vydac Proteins C4, 0.1% TFA, 206 nm	LOD: 2.1 µg/ml (3.3 s/m)	10
SK3530	Tablet		Capcellpack MG, 20 mM Ammonium acetate (pH 4.0)/CH_3CN (=60:40, v/v), 250 nm	LOD: 0.07 µg/ml	37
Paracetamol	Bulk drug, tablet	Ultrasonication	Symmetry C18, Gradient elution with a) 0.01 M H_2KPO_4 buffer (pH 3.0) and b) CH_3CN, 215 nm	LOD: 0.05 µg/ml (S/N=3)	38
Ofloxacin	Tablets	Ultrasonication	Agilent Zorbax SB-C18, Orthophospholic acid-potasium perchlorate/CH_3CN (=18:82, v/v), 294 nm	LOD: 0.03 µg/ml (3.3 s/m)	39
Famciclovir	Tablets	Ultrasonication	Symmetry Shield RP18, 50 mM Phophate buffer (pH 3.05)/MeOH (=50:50, v/v), 242 nm	LOD: 0.19 µg/ml	40
Paclitaxel			Phenomenex Cursoil, Gradient elution with a) 40% CH_3CN and b) 70% CH_3CN, 228 nm		41
Atorvastatin, lovastatin, paravastatin, rosuvastatin, simvastatin	Tablets	Ultrasonication	Intersil ODS 3V, Gradient elution with mixtures of 0.01 M ammonium acetate (pH 5.0)/MeOH/CH_3CN, 237 nm	LQD: 0.1 µg/ml (RSD<20%)	42
Paclitaxel	Lipophilic polymer complex	TLC	Purospher STAR Si, 1-2.5% Methanol in dichloromethane, 240 and 254 nm	LOD: 1.4 µg/ml	43
Donepezil	Tablets	Ultrasonication	Microsorb-MV RP-18, 20 mM Phosphate buffer (pH 2.7)/MeOH/TEA (=50:50:0.5, v/v/v)		44
Phenylpropanolamine, phenylephrine, chlorpheniramine, caffeine	OTC drug	Ultrasonication	Daisopak SP-120-5-ODS BP, 0.1 M CH_3COOH/MeOH/CH_3CN/TEA (=60:20:20:0.6, v/v/v/v), 254 nm	LOD: 0.48 µM (Phenylpropanolamine), 0.13 µM (phenylephrine), 0.14 µM (chlorpheniramine and caffeine)	45

Table 1. contd.

Medicament	Formulation	Pretreatment	HPLC conditions	Notes	Ref
Amoxicillin, ampicillin, cephalexin	Capsule		Chromolith Flash RP-18e, 0.1 M Acetate buffer (pH6.2) /MeOH (=90:10, v/v), 250 nm		23
[EC detection]					
Levodopa methyl ester	Bulk drug, capsule		Zorbax Bonus RP C18, 50 mM H_2KPO_4 (pH 3.2)/MeOH/CH_3CN (=90:8:2, v/v/v) containing 5 mM SHS, 5 mM EDTA and 5 mM NaCl	LOD: 6 ng/ml (S/N=3)	25
[MS detection]					
Paclitaxel	Liposome formulation	Solid-phaese microextraction	Agilent C8-silica column, 2 mM Ammonium acetate (pH 4.85)/ CH_3CN (=50:50, v/v),	APEI-MS	13
[FL detection]					
Voglibose	Tablet	Ultrasonication	Cosmosil 5NH_2-MS, 30 mM NaH_2PO_4 (pH 6.5)/ CH_3CN (=2:1, v/v), Ex 306 nm, Em 430 nm	LOD: 9.4 ng/ml (3.3 σ/s) Labeling reagent: taurine and sodium periodate	18
Amantadine, memantine	Tablet, capsule	Ultrasonication, liquid-liquid extraction	Purospher Star RP-18e, MeOH/H_2O (=91:9, v/v), Ex 227 nm, Em 348 nm	LOD: 0.2 nmol/injection (S/N=3) Labeling reagent: (2-naphthoxy) acetyl chloride	46
[CL detection]					
Hydrochlorothiazide, captopril		Ultrasonication	LiChrocart RP C18, 50 mM Acetate buffer (pH 3.5) containing 25 mM OSA	LOD: 5 pmol/injection (hydrochlorothiazide), 67 pmol/injection (captopril) (3σ blank) CL reagent: Ce(IV), rhodamine 6G	47

Abbreviation: TFA, trifloroacetic acid; TEA, triethylamine; SHS, sodium heptanesulfonate; OSA, sodium octane sulphonate.

Table 2. HPLC method for medicaments in biological samples.

Medicament	Matrix	Pretreatment	HPLC conditions	Notes	Ref
[UV detection]					
Levomepromazine, clozapine	Human plasma (250 µl)	SPE	Phenomenex C8, 34 mM phosphate buffer (pH 2.0) containing 0.3% TEA /CH_3CN (=71:29, v/v)	LOD: 3ng/ml (levomepromazine), 7 ng/ml (clozapine) (3µof baseline noise)	11
Tenatoprazole	Dog plasma (200 µl)	Liquid-liquid extraction	Diamonsil C18, 10 mM H_3KPO_4 (pH 4.7)/CH_3CN (=70:30, v/v), 306 nm	LOQ: 0.02 µg/ml (RSD<20%)	55
Sodium ferulate	Dog plasma (400 µl)	Deproteination	Kromasil C18, 0.5 % CH_3COOH/ CH_3CN (=80:20, v/v), 320 nm	LOD: 6.15 ng/ml (S/N=3)	56
ADKZ	Rat plasma (200 µl)	Liquid-liquid extraction	Elite HPLC C18, 18 mM TEA (pH 3.2)/CH_3CN (=35:65, v/v), 230 nm	LOQ: 0.02 µg/ml (S/N=10)	57
Paclitaxel	Human and rat plasma and serum (100 µl)	Liquid-liquid extraction	Inertsil ODS-3, 50% CH_3CN solution, 230 nm	LOD: 3-7.5 ng/ml (S/N=3)	58
Aspirin, salicylic acid	Human serum (100 µl)	Deproteination and SPE	Develosil ODS-5, 0.7 mM Phospholic acid (pH 2.5)/MeOH (=50:50, v/v), 240 nm	LOD: 114 ng/ml (aspirin), 38 ng/ml (salicylic acid) (S/N=3)	59
Carboplatin	Human plasma	Ultrafiltration	Inertsil NH_2, 5 mM Sodium perchlorinate buffer (pH 2.4)/MeOH/CH_3CN (=10:15:75, v/v/v), 230 nm	LOQ: 0.1 µg/ml (S/N=3)	19
Enoxacine, ofloxacin, norfloxacin, ciprofloxacin, enrofloxacin	Chicken blood (50 µl)	Deproteinization	Inertsil C18, 0.4 M Citric acid/MeOH/CH_3CN (=87:9:4, v/v/v), 275 nm	2 ng/ml (Enoxacine, norfloxacin, ciprofloxacin); 0.4 ng/ml (ofloxacin); 4 ng/ml (enrofloxacin)	15
Ketoprofen, naproxen, fenoprofen, flurbiprofen, ibuprofen, Diclofenac	Human plasma (100 µl)	Deproteinization	Daisopak SP-120-5-ODS-BP, 0.1 M Acetate buffer (pH 3.5)/MeOH/CH_3CN (=40:25:35, v/v/v), 255 nm, 240nm and 220 nm	LOD: 12-75 ng/ml (S/N=3)	60
Donepezil	Human plasma (1 ml)	Liquid-liquid extraction	STR-ODS-II, 0.02 M Phosphate buffer/6 M perchloric acid/CH_3CN (=59.5:0.5:40, v/v/v), 315 nm	LOQ: 3 ng/ml	
[EC detection]					
Erythromycin, dirithromycin, tylosin, tilmicosin, spiramycin, josamycin, kitasamycin, rosamicin, roxithromycin, oleandomycin	Human urine (500 µl)	Liquid-liquid extraction	SymmetryShield RP8, Gradient elution with a) 1 M phosphate buffer (pH 6.7)/H_2O/CH_3CN (=12.5:687.5:150, v/v/v) and b) 1 M phosphate buffer (pH 6.7)/H_2O/CH_3CN (=12.5:237.5:600, v/v/v)	LOD: 0.04 µg/ml (S/N=3)	61

Table 2. contd.

Medicament	Matrix	Pretreatment	HPLC conditions	Notes	Ref
Celenbuterol	Pig liver	Liquid-liquid extraction	ODS Hypersil, Gradient elution with a) 50 mM phospholic acid and 30 mM TEA (pH 4.0) and b) MeOH/CH_3CN (=45:30, v/v)	LOD: 1.2 ng/g (S/N=3)	62
5-Hydroxyoxindole	Human serum (300 µl)	SPE	Symmetry-Shield RP18, 0.13 M Sodium phosphate buffer/CH_3CN (=96:4, v/v) containing 0.2 mM SDS and 0.2 mM nitrilotriacetic acid (pH 3.4)	LOD: 0.5 nM (S/N=3)	63
Vitamin K	Human urine (50 µl)	SPE	Hypersil-Keystone Hypurity C18, 0.05 M Acetate buffer (pH 3.0) containing 0.1% EDTA/MeOH (=60:40, v/v)	LOD: 3.5 fmol/injection (5 × baseline noise)	64
Metoclopramide, hydrochlorothiazide, imipramine, diclofenac	Human serum or plasma (1 or 0.5 ml)	Liquid-liquid extraction	Nucleosil 100 C18, Mixture of phosphate buffer and MeOH	LOD: 0.8-5.0 ng/ml (10 SD of blank)	30
[MS detection]					
Paclitaxel and metabolites	Human plasma (200 µl)	Liquid-liquid extraction	Zorbax Extend C18, 10 mM NH_3 *aq.* solution/MeOH (=30:70, v/v)	LOQ: 0.25 ng/ml (RSD<20%) ESI-MS/MS	65
Triptolide, wilforlide A, triptonide	Human plasma (0.5 ml)	SPE	Macherey-Nagel C18, 2.7 mM Formic acid containing 10 mM ammonium acetate/CH_3CN (=55:45, v/v)	LOD: 0.25-0.4 ng/ml (S/N=3) ESI-MS	66
Indapamide	Human plasma (0.2 ml)	Liquid-liquid extraction	Shim-pack C18, Gradient elution with a) 1 µM ammonium acetate and 0.4 µM TEA, b) MeOH	LOD: 0.2 ng/ml (S/N=3) ESI-MS, SIM mode	67
Cyclovirobuxine D	Human plasma (1 ml)	Liquid-liquid extraction	Hanbon Lichrospher 5-C18, 30 mM Ammonium acetate containing 1% formic acid/MeOH (=48:52, v/v)	LOQ: 10.11 pg/ml (RSD<20%) APCI-MS, SIM mode	68
Glucosamine	Human plasma (490 µl)	Deproteinization	Shodex® Asahipak NH_2 P50- 2D, Gradient elution with H_2O and CH_3CN	LOD: 10 ng/ml (S/B=5, RSD<20%, Accuracy<±20%) ESI-MS/MS	20
Gambogic acid	Human plasma (1 ml)	Liquid-liquid extraction	Hanbon Lichrospher 5-C18, H_2O/THF/CH_3CN (=7:23:70, v/v/v)	LOQ: 3.1 ng/ml (RSD<20%, Accuracy<±20%) APCI-MS, SIM mode	69
Pseudoephedrine, cetirizine	Human plasma (200 µl)	Deproteinization	Lichrospher C8, Gradient elution with 0.13% formic acid and MeOH	LOQ: 1.0 ng/ml (RSD<15%, Accuracy<±15%) ESI-MS/MS, SIM mode	16
Sodium ferulate	Human plasma (1 ml)	Liquid-liquid extraction	Agilent Zorbax SB-C18, 0.05% CH_3COOH/MeOH (=60:40, v/v)	LOD: 3 pmol/ml (S/N=3) ESI-MS, SIM mode	70
Mitiglinide	Human plasma (200 µl)	Liquid-liquid extraction	Shimadzu VP-ODS, 10 mM CH_3COOH/MeOH (=35:65, v/v)	LOD: 1.42 pmol/ml (S/N=3) ESI-MS, SIM mode	71

Table 2. contd.

Medicament	Matrix	Pretreatment	HPLC conditions	Notes	Ref
Donepezil	Dog plasma (400 µl)	Column switching	Precolumn: Shimpack Mayi-ODS, H_2O/ammonium acetate/CH_3CN mixture Separation column: Symmetry C18, H_2O/TFA/CH_3CN mixture	LOD: 1 pg/ml (S/N=3) ESI-MS, SIM mode	52
[FL detection]					
Nabumetone and metabolites	Human plasma (0.5 ml)	SPE	LiChrospher®100 RP-C18, H_2O/CH_3COOH/CH_3CN (=55:1:45, v/v/v), Ex 230 nm, Em 350 nm	LOQ: 0.01-0.02 nmol/ml	72
Donepezil	Human, rat plasma, blood brain microdialysate	Liquid-liquid extraction for plasma	Develosil Combi-RP-5, Citric acid-HNa_2PO_4 (pH 6.0)/CH_3CN (73:32, v/v) containing 3.5 mM sodium octanesulfonate, Ex 325 nm, Em 390 nm	LOD: 0.2-2.1 ng/ml (S/N=3)	17
Nabumetone	Human plasma (0.5 ml)	Liquid-liquid extraction	Nova-Pak C18, 0.02% TEA (pH 7)/CH_3CN (=50:50,v/v), Ex 230 nm, Em 356 nm	LOD: 0.05 ng/ml (S/N=3)	73
DB-67	Mouse plasma (200 µl)	Deproteinization	Nova-pak C18, 0.15 M Ammonium acetate containing tetrabutylammonium dihydrogen phosphate (pH 6.5)/CH_3CN (=65:35, v/v), Ex 380 nm, Em 560 nm	LOD: 1 ng/ml	74
Doxazosin	Human plasma (300 µl)	Liquid-liquid extraction	Capcell Pak C18, 10 mM Perchloric acid and 1.8 mM SHS/MeOH (=50:50, v/v), Ex 330 nm, Em 395 nm	LOQ: 0.3 ng/ml (RSD<20%)	75
Mazindol and its metabolite	Mouse brain and plasma (100 µl)	Liquid-liquid extraction	Daisopak-SP-120-5-ODS AP, 0.1 M CH_3COOH/MeOH/CH_3CN (=50:4:46, v/v/v) containing 6%THF	LOD: 2.8 fmol/injection (plasma), 3.5 fmol/injection (brain)(S/N=3) Labeling reagent: DIB-Cl	76
Haloperidol	Human serum (500 µl)	Liquid-liquid extraction	Lichrosorb Si60, H_2O/CH_3CN (10:90, v/v) containing 0.01% TEA and 0.02% CH_3COOH, Ex 330 nm, Em 420 nm	LOD: 0.2 ng/ml (S/N=3) Labeling reagent: DPA	22
Bromperidol			Mightysil RP-18GP ODS, 0.1 % TFA/EtOH/CH_3CN (=40:20:40,v/v/v), Ex 470 nm, Em 540 nm	LOD: 3 ng/ml (S/N=3) Labeling reagent: NBD-F	77
Alendronate sodium	Human plasma (3 ml)	Deproteinization, SPE	Capcell Pak C18, Gradient elution with a) MeOH/CH_3CN (=50:50, v/v) and b) 25 mM citric acid-sodium pyrophosphate, Ex 260 nm, Em 310 nm	LOQ: 1 ng/ml Labeling reagent: FMOC	78
[CL detection]					
Artemisinin	Human serum (500 µl)	Liquid-liquid extraction	Supelco Discovery HS C_{18}, 20 mM Imidazole-HNO_3 (pH 8.5) containing 70% CH_3CN	LOD: 17.5 ng/ml (S/N=3) PO-CL reagent: oxalate, DNPO; fluorophore, TMP UV irradiation	79

Table 2. contd.

Medicament	Matrix	Pretreatment	HPLC conditions	Notes	Ref
Atanolol, metoprolol, sotalol, nadolol, pindolol	Human urine	Four-fold dilution	XTerra RP18, 10 mM Borate buffer (pH 9.0) containing 15% CH_3CN	LOD: 0.5 μM (Atanolol), 0.08 μM (metoprolol), (S/N=3) ECL reagent: $Ru(bpy)_3^{2+}$	80
Indomethacin	Human urine (100 μl)	Dilution	Nucleosil RP-C_{18}, $MeOH/H_2O/CH_3COOH$ (=67:33:0.1, v/v/v)	LOD: 8 ng/ml (S/N=3) CL reagent: Mn (III)	81

Abbraviation: TEA, triethylamine; SDS, sodium dodecylsulfonate; THF, tetrahydrofuran ; DIB-Cl, 4-(4,5-diphenyl-1*H*-imidazol-2-yl)benzoyl chloride; NBD-F, 4-fluoro-7-nitro-2,1,3-benzoxadiazole: FMOC, 9-fluorenylmethyl chloroformate; DPA, 4-(4,5-diphenyl-1*H*-imidazole-2-yl)phenylbronic acid; DNPO, bis(2,4-dinitrophenyl)oxalate; TMP, 2,4,6,8-tetrathiomorphorinopyrimido[5,4-*d*]pyrimidine.

Table 3. Chiral analysis methods of medicaments.

Medicament	Matrix	Detection	Pretreatment	HPLC conditions	Notes	Ref
Levodopa methyl ester	Bulk drug, capsule	UV		Chiralpak AD-H, *n*-Hexane/EtOH/2-propanol/diethylamine (=600:300:98:2, v/v/v/v), 280 nm	LOD: 0.060 µg/ml	25
Acetylcholinesterase inhibitors		UV		Chiralcel OD, *n*-Hexane/2-propanol (=99:1, v/v), 225 nm or 265 nm		21
Donepezil	Rat plasma (100 µl)	UV	Deproteinization	Chiralcel OD, *n*-Hexane/isopropanol /TEA (=87:12.9:0.1, v/v/v), 268 nm	LOD: 20 ng/ml (S/N=3)	27
Fluoxetine		FL with labeling		Chiralpak AD, Gradient elution with isopropyl alchol/hexane Chiralcel OD, Gradient elution with a) hexane/THF and b) *n*-hexane/THF/ CH_3CN	Labeling reagent: DBD-COCl (Ex 454 nm, Em 562 nm), NBD-COCl (Ex 478 nm, Em 537 nm)	26
Flurbiprofen	Rat serum (100 µl)	UV	Liquid-liquid extraction	Chiralpak AD-RH, H_2O/CH_3CN/ phospholic acid (=60:40:0.05, v/v/v), 247 nm	LOQ: 0.05 µg/ml	29
Troglitazone	Rat plasma (100 µl)	ECD	Liquid-liquid extraction	Chiralcel OJ-RH, $MeOH/CH_3COOH$ (=1000:1, v/v) containing 50 mM $LiClO_4$	LOD: 1.3 fmol/injection (S/N=3)	30
Warfarin	Human plasma (200 µl)	MS/MS	Automated liquid extraction	Chiralpak AD-H, $EtOH/CO_2$ (=30:70, v/v)	LOD: 13.6 ng/ml APEI	31

Abbreviation: TEA, triethylamine; THF, tetrahydrofuran.

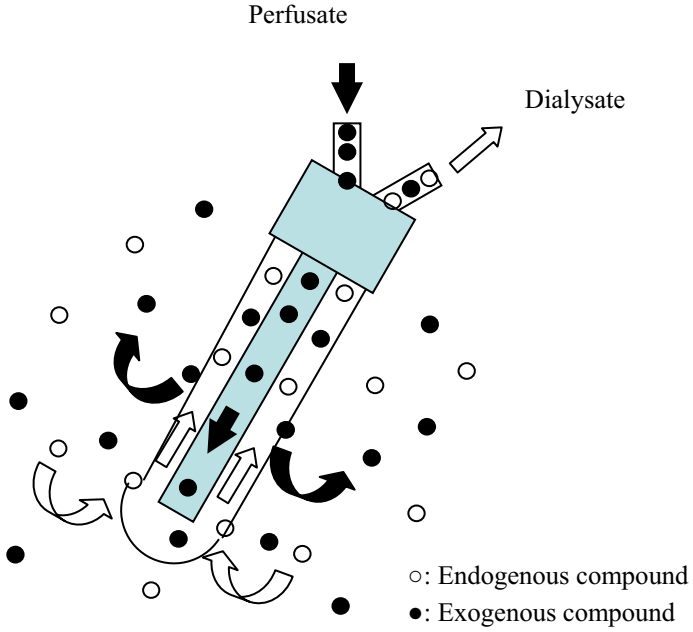

Figure 1. A microdialysis probe system

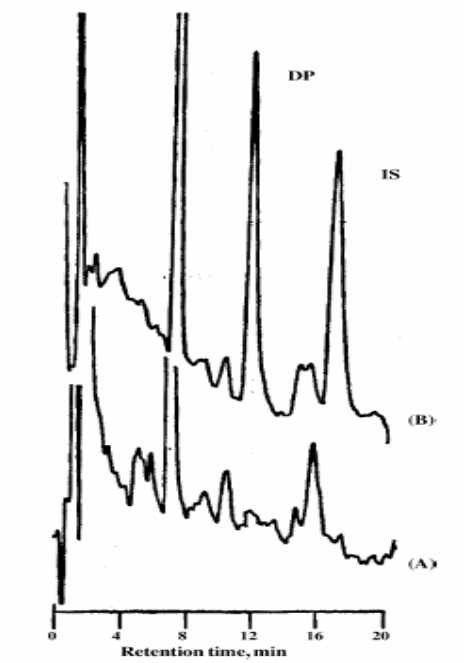

Figure 2. Chromatograms obtained from human plasma (A) and spiked with 25 nM of DP (B)
The detector sensitivity in (A) is two times higher than in (B). Printed from Ref. 17 with permission from Elsevier Sciences, B.V.

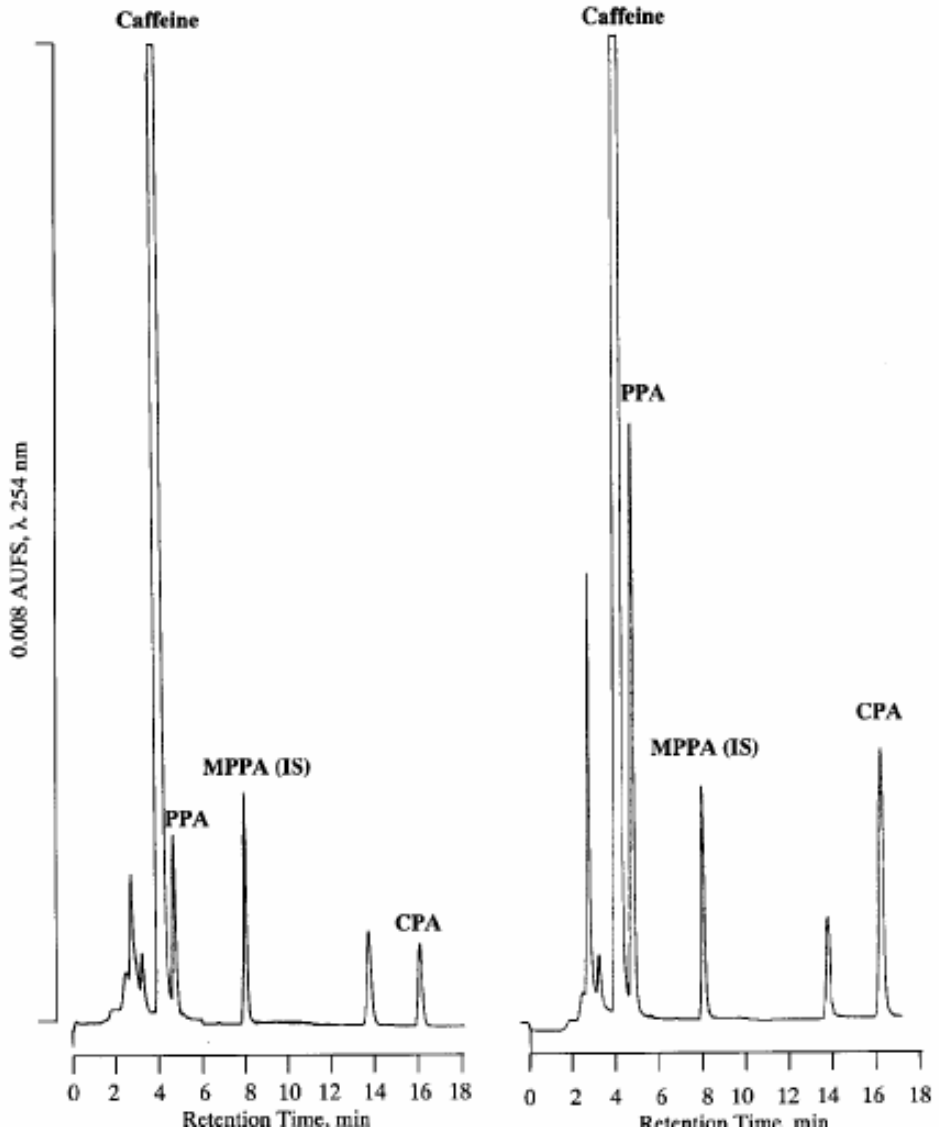

Figure 3. Chromatogram obtained from the soft capsule preparation

(A) Before spiking, with the concentrations 151, 100 and 3.8 mM of caffeine, PPA and CPA. MPPA concentration is 10 mM. (B) The sample spiked with 200 and 7.6 mM of PPA and CPA, respectively. Printed from Ref. 45 with permission from John Wiley & Sons, Ltd.

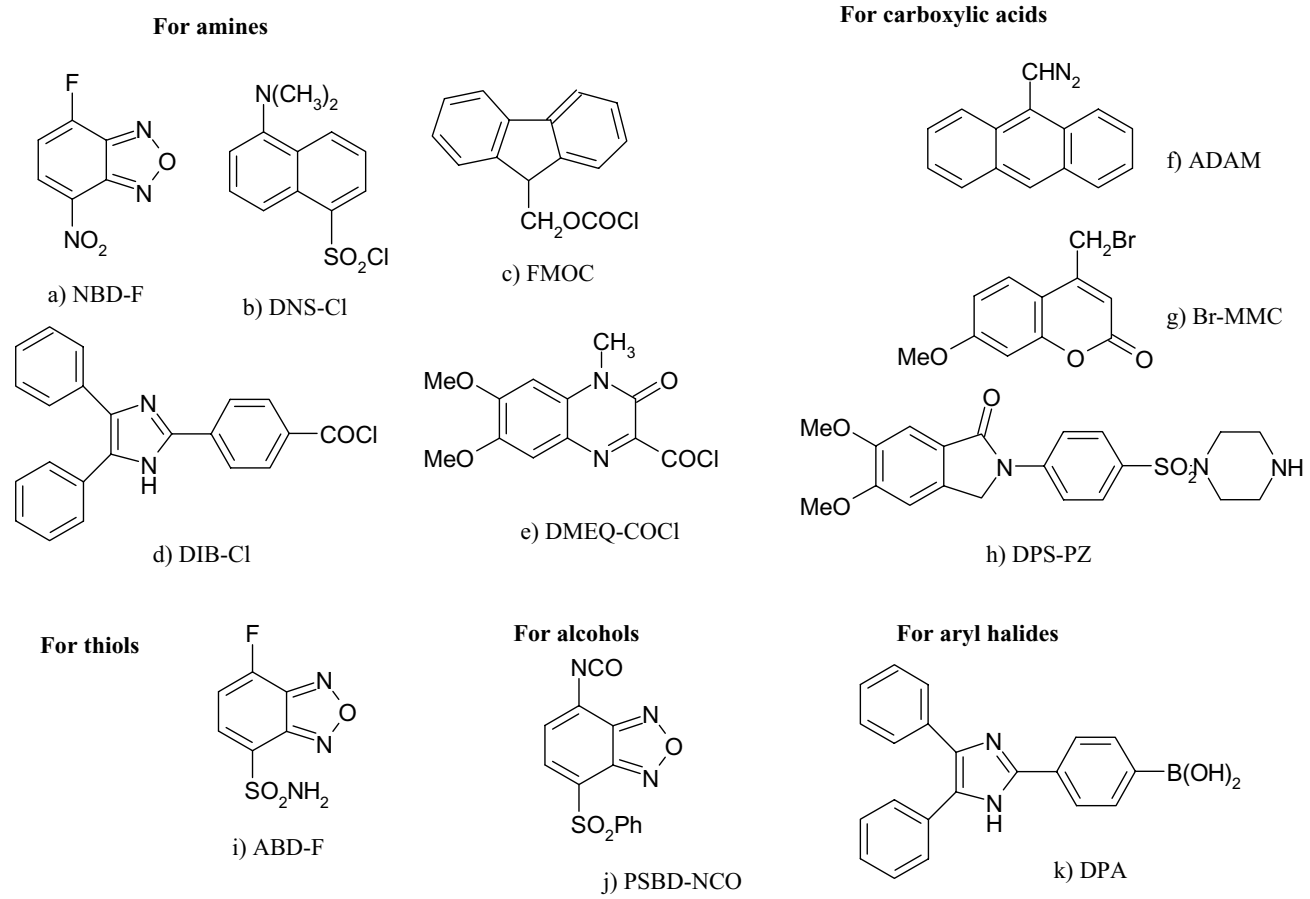

Figure 4. Representative FL labeling reagents.

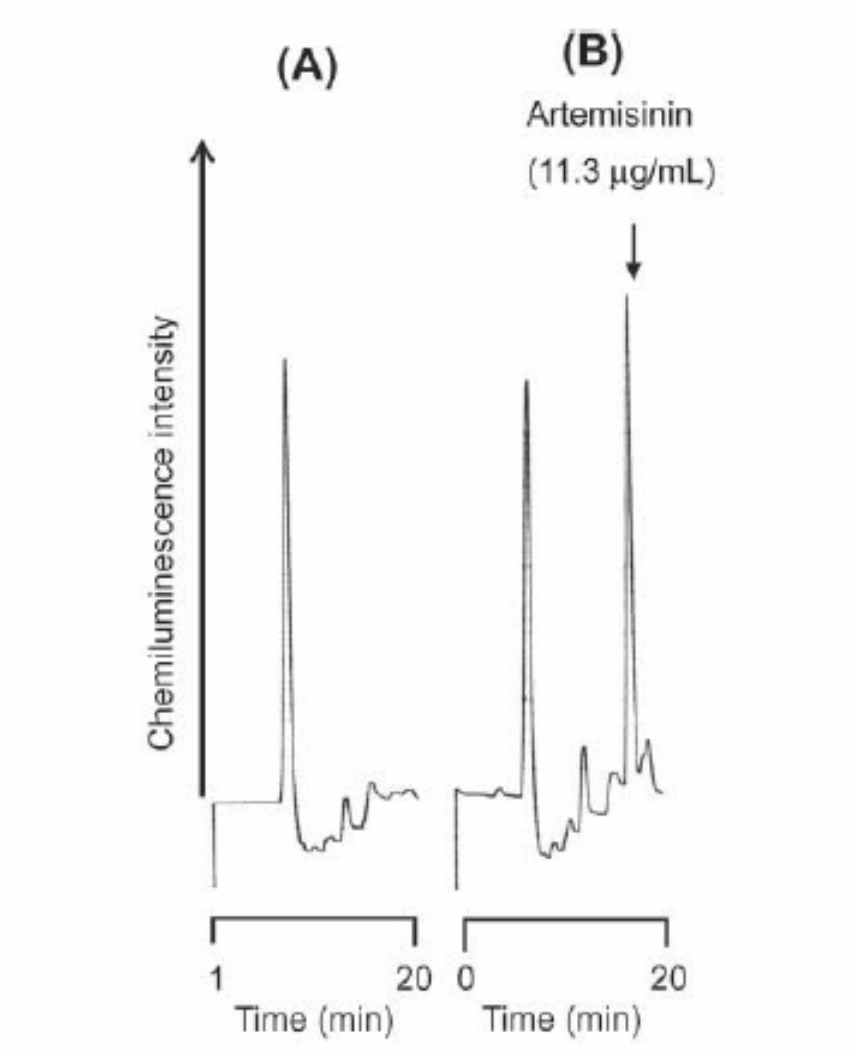

Figure 5. Chromatograms of (A) human serum and (B) human serum spiked with artemisinin
Spiked artemisinin concentration, 40 µmol/L (11.3 µg/mL). Conditions, mobile phase, 20 mmol/L imidazole buffer (pH 8.50) and acetonitrile (30:70, v/v); CL reagent, 0.50 mmol/L DNPO and 1.50 µmol/L TMP in acetonitrile, flow rate of 1.0 mL/min; reaction coil length, 6.0 m. Printed from Ref. 79 with permission from John Wiley & Sons, Ltd.

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الطرق الحديثة لتحديد الأدوية في توليفات الأدوية والعينات البيولوجية باستخدام كروماتوغرافيا السوائل عالية الأداء

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ملخ ص

لقد حدث تقدم ملموس في أداء أجهزة كروماتوغرافيا السوائل عالية الأداء (HPLC) ونتيجة لذلك لعبت هذه الأجه زة دوراً فعالاً في تحليل الأدوية وتوليفاتها والعينات البيولوجية المتعلقة بها.

إن طرق الكشف المختلفة المستخدمة لمثل هذه الأجهزة باستخدام الأشعة فوق البنف سجية (UV) ، ومطي اف الكتل ة (MS) والفلورسنس(FL) و وكيمياء الانبعاث الليومنسية (CL) بالإضافة إلى الكشف الكهروكيميائي (ECD) والمعالجة المسبقة وطريقة الوصف للعينة يتم اختيارها بناءً على الهدف أو سبب التحليل.

اعتمد في تحليل الأدوية في توليفاتها على الكشف باستخدام الأشعة فوق البنفسجية، بينما طرق الكشف الأخرى مث ل (FL) و(MS) و (CL) و (ECD) وحساسيتها العالية تستخدم لتحليل الأدوية في العينات البيولوجية حيث تق ع ه ذه الحساسية ضمن مدى الكتل ما بين المايكروغرام والبيكوغرام.

تم في هذه المراجعة مناقشة الطرق (HPLC) المستخدمة لتحديد الأدوية في توليفاتها الدوائية والعينات البيولوجية، وعلاوة على ذلك تم التعرض للتطبيقات المتقدمة لهذه الأجهزة في مجال تحليل ودراسة وحركة وتداخلات الدواء من اجل تقييمه.

الكلم ات الدال ة: كروماتوغرافيا السوائل عالية الأداء، أدوية، عينات بيولوجية وتوليفات.

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