Focusing Review

Development and practical application of HPLC methods for medicaments and related compounds

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Abstract

HPLC methods for determination of medicaments and related compounds were developed to promote the rational use of these compounds. According to the aim of analysis, suitable hyphenated detections such as UV, fluorescence and chemiluminescence with HPLC were used. Labeling, a powerful tool to provide desirable chromatographic properties to the analytes was also utilized for sensitive determination of medicaments and related compounds in biological samples such as urine, plasma, hair and microdialysate. Furthermore, the methods could be successfully applied to evaluate drug−drug interaction or quality control of healthy foods.

These developed HPLC methods could be contribute to the practical application studies on the rational use of medicaments.

Keywords: Rational use of medicaments; HPLC; Biological sample; Hyphenated detection; Labeling

1. Introduction

"Rational use of medicaments" is one of the most considerable paradigms in the pharmaceutical region. All of pharmaceutical researches might be performed to promote this. Chromatographic analysis of medicaments in biological fluids is often required to accomplish the rational use of medicaments and related compounds [1]. Nowadays, any useful information such as quality control, pharmacokinetics, pharmacodynamics, pharmacology and toxicology of medicament are always obtained with chromatographic analysis. Therapeutic drug monitoring based on determination of medicaments is one of the representative examples.

Among the separation techniques employed for medicament or related compounds analyse such as thin−layer chromatography, gas chromatography, high−performance liquid chromatography (HPLC), capillary electrophoresis and capillary electrochromatography, HPLC and GC have been proven to be the most popular, because these can offer better sensitivity, selectivity and applicability. In addition, most medicaments and related compounds as well as endogenous components in biological samples are commonly non−volatile and polar compounds. And HPLC is become widespread, thus it is the most suitable among chromatographic techniques for their analysis.

If the target molecule does not have desirable properties for spectrometric or luminometric analysis, a labeling reaction is needed to introduce the properties in these 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 separation behavior. In our previous studies, 4−(4, 5−diphenyl−1*H*−imidazol−2−yl)benzoyl chloride (DIB−Cl) was one of the most powerful fluorescent labeling reagents and has been applied to determine the varied medicaments such as 3,4−methylenedioxymethamphetamine (MDMA) and its metabolite [2, 3], and morphine (Mor) [4]. Especially, the methods for MDMA were successfully showed applicability to the several biological samples such as human hair [3] and plasma, rat brain and blood microdialysates [2]. To date, many

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labeling reagents have been developed for each reactive functional group. Several reviews describing the labeling reagents for HPLC have been published [5−7].

On the other hands, microdialysis technique is often utilized as a sampling tool to monitor concentrations of free−form compounds or neurotransmitters in organs, and is also applied for neuropharmacological and pharmacokinetic studies [8]. A microdialysis probe consists of a small semipermeable membrane, on which analytes are recovered by passive diffusion. 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. This tool played important role in our application studies on pharmacokinetics and drug−drug interactions [2, 4, 9].

The aim of this review is to overview our current HPLC methods for determination of medicaments and related compounds (*e.g.* drugs of abuse and food components) in biological samples. Furthermore, their advanced applications for evaluation of pharmacokinetic behavior on drug−drug interaction of medicaments are described.

2. HPLC methods for determination of medicaments and their applications

Representative medicaments described in this chapter were indicated in Figure 1. The HPLC−UV detection has wide adaptability and simplicity with a relatively inexpensive instrument, although the sensitivity of the UV detection inferior to those of MS, FL or electrochemical detection. However, since the performance of UV detector has been recently improved and also the levels of medicaments in biological samples are relatively high (sub−micro g/mL level), these determination can be achieved by an HPLC−UV method. Among the medicaments, aspirin (Asp) and its metabolite, salicylic acid (SA), in human serum were determined by an HPLC−UV method [10]. A relatively small volume of serum (100 μL) cleaned up by solid phase extraction was required for determination with limit of detections (LODs) of 0.11 (Asp, signal−to−noise ratio (S/N) = 3) and 0.04μg/mL (SA), respectively. This method could be applied to determine analytes in patients' sera administered with low−dose enteric−coated Asp. A simple HPLC−UV method was developed for determination of paclitaxel, an anti−tumor agent in human and rat blood samples [11]. Paclitaxel in plasma was extracted by a simple liquid−liquid extraction with *tert*−butylmethyl ester. The LOD of paclitaxel in human plasma was 10ng/mL (at S/N=3). This HPLC method was successfully applied to pharmacokinetic studies of paclitaxel administered to rats.

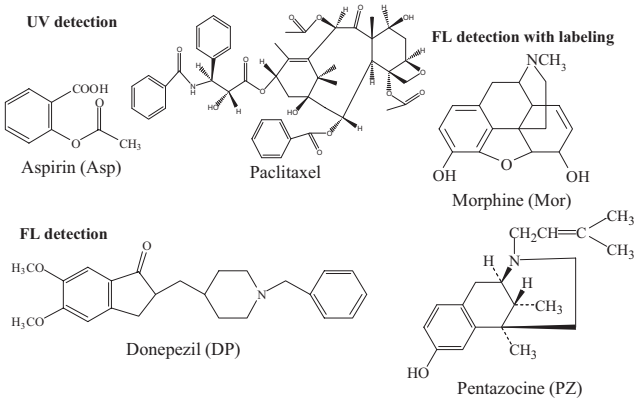

Figure 1. Target medicaments in this study.

FL detection counts the increase in photon number occurred, while UV detection measures the ratio of transmitted light that passes out through the sample solution and the original irradiation light. Therefore an HPLC−FL detection method has been favorably comparable to UV detection in regard to sensitivity and selectivity, and extensively employed for the determination of trace amounts of compounds. 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. Medicaments with their intrinsic FL can be detected by HPLC−FL without pretreatment such as fluorescent labeling. In our previous report, by utilizing intrinsic FL of donepezil (DP) hydrochloride, rapid and sensitive determination of DP in human plasma, rat plasma and microdialysis was achieved with a short C_{30} column and an isocratic elution [9]. The method provided the LOD of DP in human plasma, rat plasma and microdialysis ranged from 0.2 to 2.1ng/mL (S/N =3), and was successfully applied to plasma samples from Alzheimer's patients or rat brain and blood microdialysates after a single administration of DP. Furthermore, the possibility of interaction of Asp or clopidogrel on DP hydrochloride in rats was evaluated [12]. Pharmacokinetic parameters of DP for administration of DP (5mg/kg, i.p.) and co−administration of Asp (200mg/kg, p.o. or 200mg/kg, i.p.) or clopidogrel (5mg/kg, p.o.) groups were calculated and compared among them; no significant difference was observed.

Morphine (Mor) is most frequently used as an analgesic drug for both postoperative and cancer pain. The World Health Organization proposed a three−stage approach for the treatment of chronic pain, in which opioids including Mor are used in combination with nonsteroidal anti−inflammatory drugs (NSAIDs). Several studies have demonstrated the benefits of the combined use of Mor with NSAIDs in comparison with Mor alone [13]. These reports detailed the additive effect on pain relief caused by the different pharmacodynamics of Mor and NSAIDs. On the other hand, although pharmacokinetic interactions between Mor and NSAIDs cannot be neglected, only limited knowledge is available [14]. This might be caused by the lack of a simple and sensitive method for Mor determination. Therefore, a sensitive HPLC−FL detection method coupled with a microdialysis technique was developed for the determi-

nation of Mor in brain and blood microdialysates [4]. Mor was easily labeled with DIB−Cl under the mild reaction conditions (at room temperature for 10 min, Figure 2). The separation of DIB−Mor was carried out on a C_{18} column with CH_3CN/0.1 M acetate buffer (pH 5.4) within 14 min as shown in Figure 3. The LODs of Mor in brain and blood microdialysates at S/N=3 were 0.4 and 0.6 ng/mL, respectively. The proposed method was successfully applied to the preliminarily study of potential pharmacokinetic interaction between Mor and diclofenac which was one of the most frequently used NSAIDs [15]. No significant difference was observed for any pharmacokinetic parameters of Mor between rats administered Mor with/without diclofenac. Pentazocine (PZ) which is structurally similar to Mor, is a shortacting narcotic−antagonist analgesic and was determined by an HPLC−FL method with DIB−Cl labeling [16]. PZ extracted from hair or plasma sample was labeled and the resulted solution was cleaned up with solid phase extraction. The LODs of PZ at S/N=3 for rat hair and plasma were 0.18 ng/mg and 0.57 ng/mL, respectively. The method was successfully applied for the monitoring of PZ levels in Zucker rat hair and plasma samples after a single administration of 25 mg/kg PZ. The incorporation rate values of PZ were calculated from the following equation;

The incorporation rate = PZ concentration in black or white hair/AUC_{0-inf} of PZ in plasma

The ratios for black and white hair were 0.053 and 0.007, respectively.

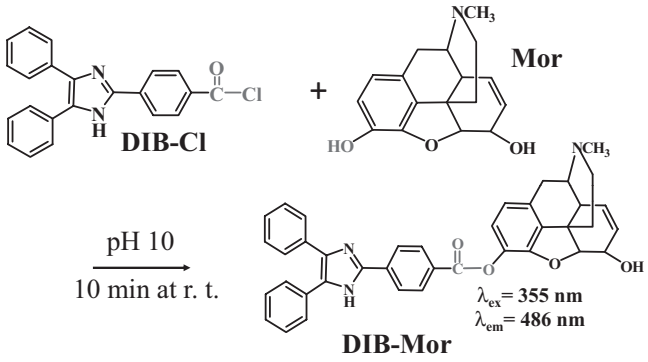

Figure 2. Labeling reaction of Mor with DIB−Cl.

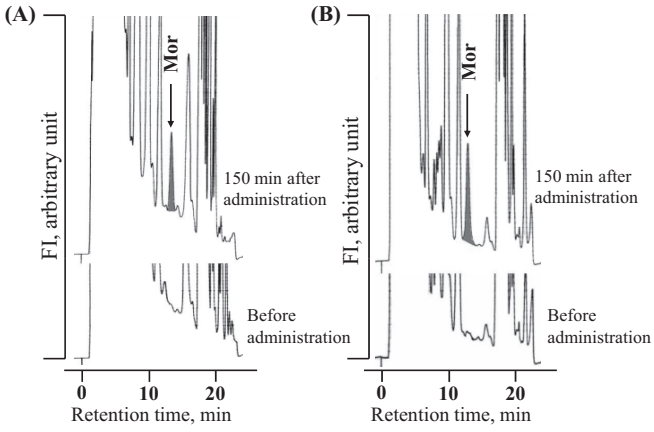

Figure 3. Chromatograms of (A) brain dialysate and (B) blood dialysate 150 min after administration of Mor.
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3. HPLC methods for determination of abused drugs and their applications

Abuse of drugs has been widespread worldwide and nowadays, drugs of abuse involving medicaments have been diversified. Therefore, development of determination methods for drugs might lead to promote “rational use of medicaments”. Among drugs of abuse, 3,4−methylenedioxymethamphetamine (MDMA), commonly called ‘ecstasy’, is an amphetamine analog used as a recreational drug among the young. It is usually used as an ecstasy tablet, and the concentration of MDMA in the tablets is variable and uncertain, and thus it is possible to take an overdose that serves toxic effects. The toxicology of MDMA and related drugs has been reviewed in detail [17]. Additionally, since an ecstasy tablet includes other components such as caffeine, ketamine, ephedrine and methamphetamine, unexpected toxicity may appear owing to drug−drug

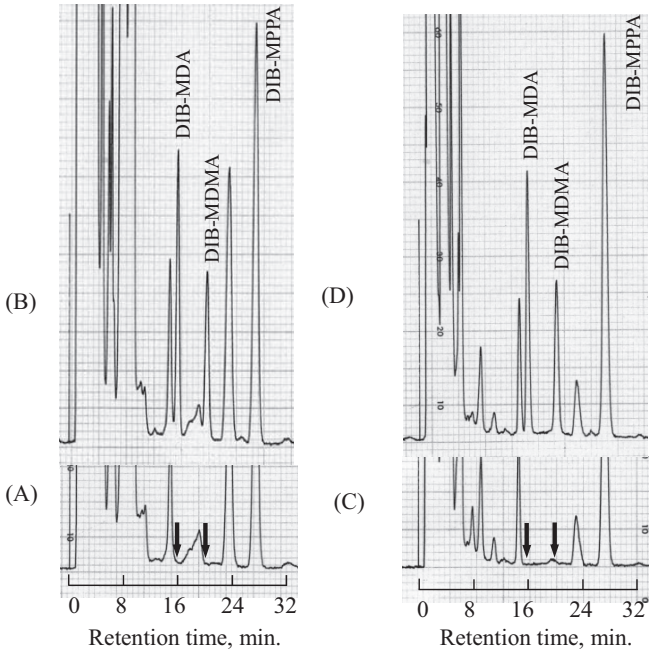

Figure 4. Chromatograms of rat blood and brain dialysates.
Samples: (A) control rat blood microdialysate spiked with 100 ng/mL of IS; (B) spiked blood microdialysate with 50 ng/mL of MDA, 100 ng/mL of MDMA and 100 ng/mL of IS; (C) control rat brain microdialysate spiked with 100 ng/mL of IS; and (D) spiked brain microdialysate with 50 ng/mL of MDA, 100 ng/mL of MDMA and 100 ng/mL of IS.
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interactions [18]. Therefore, simultaneous determination of MDMA and its metabolite 3,4−methylenedioxyamphetamine (MDA) in rat blood and brain microdialysates by HPLC−FL was developed [2]. Microdialysates were directly subjected to labeling with DIB−Cl. The DIB−derivatives of MDMA, MDA and the internal standard, 1−methyl−3−phenylpropylamine (MPPA), were isocratically separated on an ODS column using a mixture of 50 mM phosphate buffer (pH 7.0)/CH_3CN/CH_3OH/2−propanol (50:45:5:2, v/v/v/v %) as a mobile phase (Figure 4). The LODs (S/N=3) for MDA and MDMA were 1.2 and 4.2 for blood microdialysate and 1.3 and 4.8 ng/mL for brain microdialysate, respectively. Moreover, the proposed method was successfully applied for the monitoring of MDMA and MDA in rat blood and brain microdialysates, and the pharmacokinetic parameters of MDMA in the microdialysates after administration of MDMA (5 mg/kg, i.p.) with or without caffeine (20 mg/kg, i.p.) were evaluated (Figure 5). The pharmacokinetic parameters of MDMA in blood and brain are summarized in Table 1. Following the administration of MDMA with caffeine, the AUC_{0-300} (53±5 μg•min/mL) of MDMA in blood significantly increased to 91±5 μg•min/mL ($p < 0.01$), and clearance (CL) of MDMA (96±10 mL/min/kg) decreased by half to 51±4 mL/min/kg. No change of C_{max} and AUC for MDA was observed (data was not shown), which suggested that caffeine may interfere with renal clearance of MDMA. On the other hand, the C_{max} of MDMA (1009±28 ng/mL) in brain significantly decreased to 496±80 ng/mL ($p < 0.01$) and 93±21 ng/mL ($p < 0.05$). Moreover, the T_{max} and the mean residence time (MRT) of MDMA were considerably prolonged, but the elimination half−life ($T_{1/2}$) of MDMA was unchanged. These results suggested that caffeine inhibits the transportation of MDMA to brain via the blood−brain barrier, although the exclusions of MDMA from brain to blood were not influenced by caffeine. The inhibition of P−glycoprotein by caffeine was expected to be the reason for the increased level of MDMA in brain [19]. The proposed method coupled with a microdialysis technique could be concluded to be a powerful tool for pharmacokinetic interaction studies of MDMA with other drugs. Furthermore, MDMAs in several biological matrices such as urine [20] and hair [3, 21] were also determined by HPLC−FL or −CL detections.

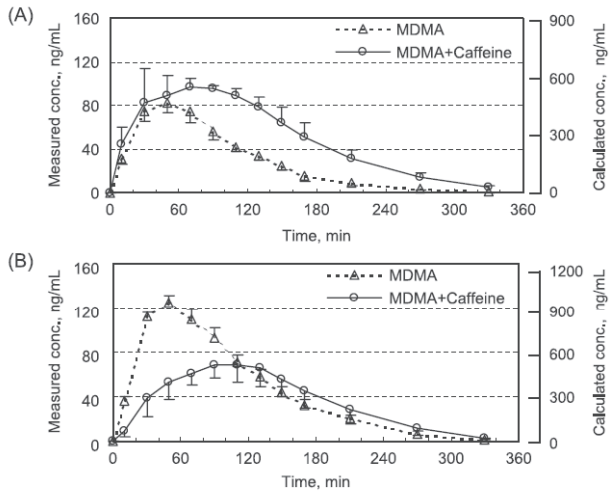

Figure 5. Concentration−time profiles of MDMA in rat blood (A) and brain (B) microdialysates after a single administration of MDMA with or without caffeine.

Data are expressed as mean±SEM (MDMA alone group, $n = 5$; MDMA with caffeine group, $n = 3$).

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Table 1. Pharmacokinetic parameters of MDMA and MDA in rat blood and brain after single administration of MDMA (5 mg/kg) with or without caffeine (20 mg/kg).

Parameters	MDMA alone (n=5)	MDMA with caffeine (n=3)
In blood		
C_{max}, ng/mL	447±50	550±45
T_{max}, min	38±5	77±24
$T_{1/2}$, min	43±8	57±6
AUC_{0-300}, mg•min/mL	53±5	91±5[b]
MRT_{0-300}, min	85±4	109±11
CL, mL/min/kg	96±10	51±4[a]
In brain		
C_{max}, ng/mL	1009±28	496±80[b]
T_{max}, min	46±4	97±24
$T_{1/2}$, min	44±5	54±9
AUC_{0-300}, mg•min/mL	118±8	93±10
MRT_{0-300}, min	92±3	112±15
CL, mL/min/kg	42±4	50±7

Data are expressed as mean ± SEM.

[a] $p < 0.05$, significantly different from MDMA alone.

[b] $p < 0.01$, significantly different from MDMA alone.

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4. HPLC methods for determination of food components and their applications

Analysis of components in healthy foods or functional foods is also important by the reasons as follows: 1) food−drug interaction has been noticed as well as drug−drug interaction. Food component analysis could provide available information to prevent such interaction. 2) There is no regulation on the quality of healthy foods in Japan. So analysis of components of these foods might contribute the preservation of their quality.

Morinda citrifolia commonly known as Noni is in high demand as an alternative medicine due to its possibilities for anti−microbial, anticancer, anti−inflammatory, antioxidant effects [22]. However, there is few available information to evaluate the effects

with quantitative data of ingredient. Scopoletin, a coumarin derivative, is one of the representative ingredients contributing to the desirable effects in Noni. Therefore, quantification of coumarin derivatives such as scopoletin, 7−hydroxycoumarin (7−HC) and 4−hydroxycoumarin (4−HC) in Noni by HPLC−UV or −FL detection was demonstrated for quality control purposes [23]. The eluent was monitored by UV and FL detectors connected succeedingly as shown in Figure 6, three standard coumarin derivatives (scopoletin, 7−HC and 4−HC) were separated within 30 min (Figure 6A). The retention times for scopoletin, 7−HC and 4−HC were 28.6, 27.2 and 10.8 min, respectively. In Figure 6B, scopoletin could be detected by both detectors. However, 7−HC was detected by only FL detector (Figure 6C). Amounts of scopoletin in Noni juices was ranging from 5.1 to 231 mg/mL (n = 12) (Table 2). And 7−HC was from 0.04 to 0.45 mg/mL, whereas no 4−HC was detected in any Noni samples examined. By a multi−wavelength FL detector, more than 95% of peak purity for coumarin derivatives in Noni sample was confirmed. Furthermore, the quenching effects of Noni products and coumarin derivatives on reactive oxygen species (ROS) were evaluated by a luminol chemiluminescent assay. Both Noni samples and coumarin derivatives dose−dependently quenched ROS such as superoxide (O_2^-), singlet oxygen ($^1O_2$), hydroxyl radical ($\cdot$OH) and peroxynitrite ($ONOO^-$). The profiling of quenching effect on ROS might be also useful for the quality control of Noni products, and due to these data, the contribution ratio of quenching effect for scopoletin could be evaluated. The EC_{50} of scopoletin for O_2^-, $^1O_2$, $\cdot$OH and $ONOO^-$ were 1.27±0.22 mg/mL, 0.68±0.04 mg/mL, >4.00 mg/mL, and 0.042±0.002 mg/mL, respectively. Based on these data, the contribution ratio of scopoletin for ROS in Noni juices was also evaluated (Table 2). The scopoletin was found to be a potent $ONOO^-$ quencher in this study. These findings might be available for the quality evaluation to control Noni products.

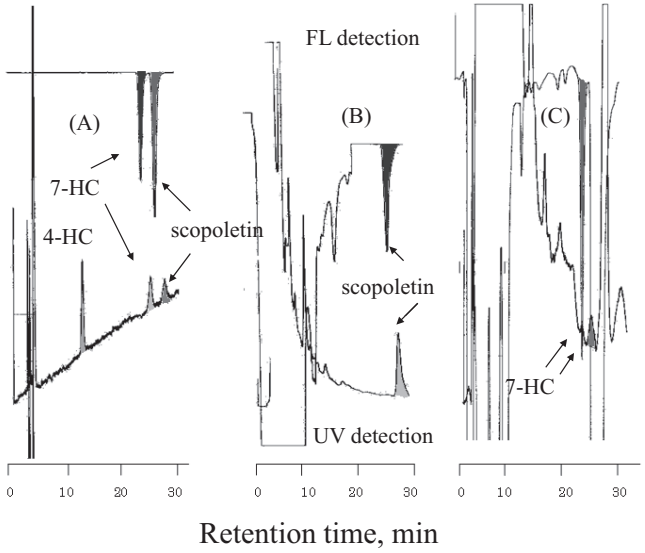

Figure 6. Chromatograms of coumarin derivatives in Noni samples.
Sample: (A) 0.1 μg/mL of standards; (B) sample K; (C) sample F. Detection: (A) 286 nm (UV) and 450 nm with 320 nm of excitation (FL); (B) and (C) 322 nm (UV) and 450 nm with 320 nm of excitation (FL).
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Curcuminoids, such as curcumin (C), demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC), are derived from the turmeric plant (*Curcuma longa* L.). These are commonly used as a dietary spice and a natural coloring agent in South and Southeast Asia. Over the years, curcumin was reported to have a wide range of pharmacological activities, including anti−inflammatory, anticancer, anti−oxide, anti−angiogenic and immunomodulatory [24]. It is believed that these desirable effects may appear upon the intake of curcuminoids or turmeric products. Since the quality of turmeric products is directly based on the amount of the curcuminoids, a reliable method for the quantitative analysis of curcuminoids in turmeric products is required. Therefore, an HPLC method using FL detection for the quantitation of curcuminoids, such as C,

Table 2. Amount and contribution ratios of quenching effect of scopoletin in Noni samples.

Sample	Concentration of scopoletin, μg/mL	Contribution ratio%			
		O_2^-	$^1O_2$	$\cdot$OH	$ONOO^-$
A	9.8	0.9	0.1	1.3	15.0
B	39.0	2.4	0.4	6.5	48.6
C	35.7	2.3	0.3	5.5	44.1
D	5.2	0.2	0.1	0.4	6.1
E	11.0	4.0	0.9	6.1	75.2
F	54.4	1.7	0.4	2.8	41.8
G	33.3	1.6	0.4	3.0	48.7
H	41.1	0.5	0.1	0.9	14.8

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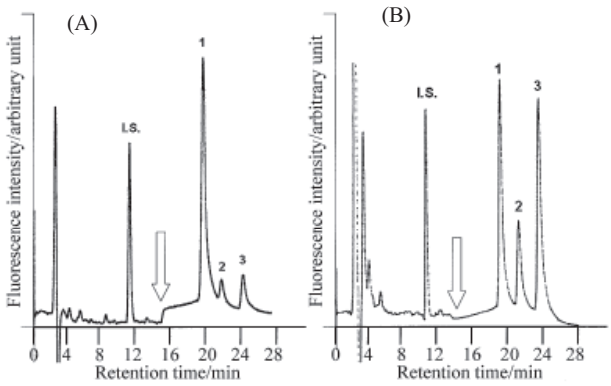

Figure 7. Chromatograms of a standard solution (A) and turmeric powder G (B).
The peak number: 1, BDMC; 2, DMC; 3, C. The arrow indicates changing of the detection wavelengths from 287 nm (λex) and 303 nm (λem) for I.S. to 426nm (λex) and 539nm (λem) for curcuminoids.
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DMC and BDMC in turmeric products was developed [25]. This method involves a simple ultrasonic extraction with methanol as a pretreatment of turmeric products. The separation of curcuminoids and 2,5−xylenol as an internal standard was achieved within 30 min on a Cadenza CD−C 18 column (250×4.6mm; i.d., 3μm) with a mixture of acetate buffer and CH_3CN (Figure 7). The instrumental LODs for C, DMC and BDMC (S/N ratio = 3) were 1.5, 0.9 and 0.09ng/mL, respectively. The proposed method was successfully applied to determine curcuminoids in commercial turmeric products, such as turmeric powders, a tablet, a dressing, a beverage, tea, and crude drugs.

5. Conclusion

By using hyphenated detections such as UV, FL and CL detections, sensitive and selective HPLC methods for determination of medicaments and related compounds were developed. FL labeling and microdialysis sampling in these methods could be powerful tools for sensitive, selective, simple and rapid determination of analytes. Furthermore, applicability of these methods was demonstrated to estimate drug−drug interaction. From now on, we will perform the research based on the development of analytical methods using chromatographic techniques and practically contribute to “rational use of medicaments”.

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