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HPLC Analysis of Camptothecin and 10-Hydroxycamptothecin in *Camptotheca Acuminata* Decne Leaves*

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Abstract: An HPLC method for the simultaneous determination of CPT and HCPT in *Camptotheca acuminata* Decne. leaves was established. Chromatography was performed with a sinochrom ODS column, monitored at 266 nm and the mobile phase was gradient H_2O/CH_3OH. The solvent gradient program was as follows: methanol-water increased linearly from 40% to 50% at the first 6 min and retained for 2 min with 50%, then increased linearly to 70% in the next 32 min. The average recoveries of CPT in three levels were 100.02%, 98.94% and 99.25%, The average recoveries of HCPT were 99.13%, 99.66% and 98.97%. Using this assay, CPTand HCPT in leaves of *Camptotheca acuminata* Decne. in different seasons were determined. The results showed that the accumulation of CPT and HCPT presented evident relativity in the different growth stages.

Key words: HPLC; *camptotheca acuminata* Decne.; leaves; camptothecin(CPT); 10-hydroxy-camptothecin (HCPT)
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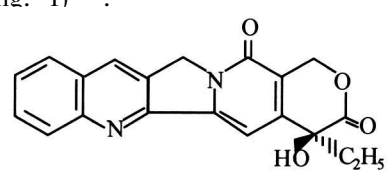
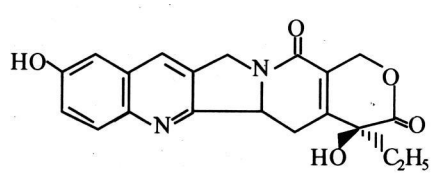
1 Introduction

Camptothecin(CPT) and 10-hydroxy camptothecin(HCPT) are two significant secondary metabolites belonging to terpenoid indole alkaloid produced by the *Camptotheca acuminata* Decne., a tree native to China (fig. 1)[1].



Camptothecin(CPT)



10-hydroxycamptothecin (HCPT)

Fig. 1 Structures of Camptothecin(CPT) and 10-Hydroxy Camptothecin(HCPT)

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They possess strong anti-cancer bioactivity and the mechanisms as well as modes of action against cancer is unique which inhibit topoisomerase I, an enzyme closely linked with cell division[2-6]. Among many CPT and HCPT analogues, irinotecan and topotecan have been approved by United State Food and Drug Administration (FDA) for application against refractory colon-rectum cancer, small-cell lung cancer and ovarian cancer[7-12]. Several other analogues are in the process of clinical test[13-14].

In recent years, several HPLC methods have been reported to applianalyze CPT drugs in *Camptotheca acuminata* Decne[15-20], but only a few of them dealt with the simultaneous of CPT and HCPT and analytical method for the two metabolites reported only in fruit and seed of *Camptotheca acuminata* Decne[18-20].

In this paper, we set out to develop a method for simultaneous determination of CPT and HCPT in leaves by HPLC. The method was successfully applied to analyze CPT and HCPT contents in leaves from different seasons in Guiyang city (China).

2 Experiment

2.1 Reagents and Materials

The methanol was of HPLC grade; water was ultrapure water; all others were analytical grade.

The CPT and HCPT standard samples were purified by researcher Xin-yi Luo and identified by Ecole Normale Superieure in Paris. Their purity was both 98%.

Four-year old *Camptotheca acuminata* Decne. in the arboretum of Guizhou normal university (Guizhou, China) was selected for the analytical sample. The leaf samples to determine the levels of CPT and HCPT were collected in different seasons. All the samples were dried at 60 till constant, crushed and passed through a 60 mesh screen sieve.

2.2 Apparatus and Chromatographic Conditions

Chromatography were performed using a P230P HPLC system (Elite, Dalian, China) consisting of a P230 gradient pump, a UV230+ UV-VIS detector and a Rheodyne MX7925 injector. The analytical column used was sinochrom ODS-BP (250 mm 4.6 mm I.D., 5.0 m). The separation was performed using a solution of methanol/distilled water mixture with a gradient elution (methanol concentration increased linearly from 40% to 50% during the first 6min and stayed for 2 min with 50%, then increased linearly to 70% in the next 32 min). The flow-rate was 1 mL/min. The determination was done at 266 nm and 25 for column.

2.3 Standard Solutions

Standard solutions of CPT and HCPT at 200 and 20 mg/L were prepared in methanol/N-N-dimethylformamide (90 10, v/v). Working standard solutions were prepared by diluting these stock solutions with the methanol/N-N-dimethylformamide mixture to achieve CPT standard concentrations of 2, 20, 60, 120, 200 mg/L and HCPT of 0.1, 2, 4, 6, 10 mg/L, respectively. A 20 L volume of each standard solution was injected in triplicate onto the HPLC column. The calibration graphs were constructed by plotting the peak areas of CPT and HCPT versus their concentrations.

2.4 Extraction and Assay Procedures

The optimal solvent for the simultaneous extraction of CPT and HCPT from *C. acuminata*. *D*. was determined. Testing was done using alcohol, methanol, 70% alcohol and 70% methanol.

5 g of the leaves powder was placed into 50 mL flask, with 45 mL of the solvent being tested then ultrasonically extracted at 50 for 60 min. After cooling to room temperature, this solution was transferred to a volumetric flask and 5 mL solvent was added to the scale. Finally it was filtrated through 0.45 m Millex syringe filters for HPLC analysis.

2.5 Recovery

Nine leave samples whose content was known precisely were weighed; every set was 1 g, and divided into three groups. The recoveries of CPT and HCPT were calculated by standard addition method.

2.6 CPT and HCPT Contents in Different Seasons

CPT and HCPT contents in *Camptotheca acuminata* Decne. leaves from different seasons were determined by the above analyzing method.

3 Results and Discussion

3.1 Chromatograms

The representative chromatograms obtained in our study were shown fig. 2 and fig. 3.

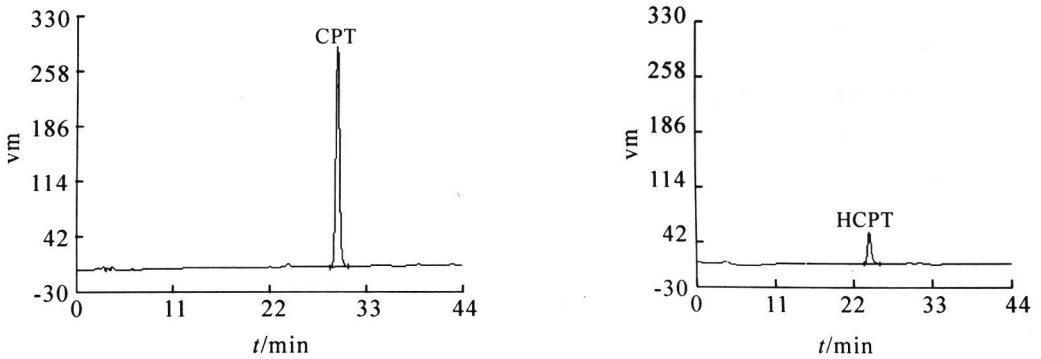

Fig. 2 HPLC Chromatogram of CPT and HCPT Standards

The retention times of CPT and HCPT were approximately 29.47 and 23.59 min, respectively. The reports about simultaneous determination methods of CPT and HCPT by HPLC are relatively few in now days. are many interfering components in extractive because of metabolism prosperity of leaves in some month of a year. Therefore, 10-hydroxycamptotheca can t be well separated with neighbor impurity refering to reported conditions. However, the separating condition was greatly improved through selecting proper condition of gradient elution. Assays of *Camptotheca acuminata* Decne leaf showed there were no interfering peaks, CPT and HCPT can be separated satisfactorily in thirty-five minutes.

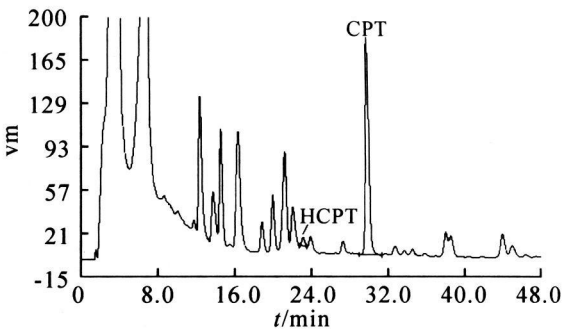

Fig. 3 HPLC Chromatogram of a Leaf Sample in July

The average recoveries of CPT in three levels were 100.02%, 98.94% and 99.25% and that of HCPT were 99.13%, 99.66% and 98.97% (Table 1). Repeatability was determined by five-fold analysis of a 4 mg/L standard solution and the RSD of CPT and HCPT were 1.76%, 1.55% respectively.

Table 1 Recovery Test (n= 3)

Sample	Added/mg	Average recovery/%	RSD/%	Sample	Added/mg	Average recovery/%	RSD/%
	0.5	100.02	0.60		0.5	99.13	0.52
CPT	1.0	98.94	0.51	HCPT	1.0	99.66	0.47
	1.5	99.25	0.37		1.5	98.97	0.31

3.2 Linearity

The calibration graphs for CPT and HCPT were linear from 4 to 200 and from 0.1 to 10 mg/L, re-

spectively. The means of their calibration graphs yielded the following equations: $y = 47.61x + 134.77$ for CPT, R= 0.999 6, and $y = 68.552x + 32.138$ for HCPT, R= 0.999 8, in which y is the peak area of CPT or HCPT to and x is the concentration (mg/L) of CPT or HCPT.

3.3 Optimal Solvent for the Sample Preparation

Optimal solvent for simultaneous extraction of CPT and HCPT from *C. acuminata D.* leaves were studied. The result shows that extracting with 60% methalol solution have a higher extraction recovery of CPT and HCPT (Table 2).

Table 2 Extraction Recovery of CPT and HCPT with Different Solvents

solvent	Extraction recovery of CPT/%	Extraction recovery of HCPT/%	solvent	Extraction recovery of CPT/%	Extraction recovery of HCPT/%
alcohol	0.071	0.001	70% alcohol	0.090	0.002
methanol	0.073	0.002	70% methanol	0.094	0.002

3.4 CPT and HCPT Contents in Different Seasons

CPT and HCPT contents in different seasons were analyzed by defined assay and the results were shown in figure 4, 5, 6 and 7.

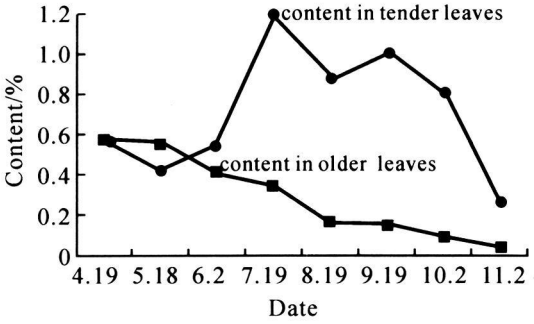

Fig. 4 Vanation of CPT Concent in Leaves of Camptotheca Acuminata in Different Months

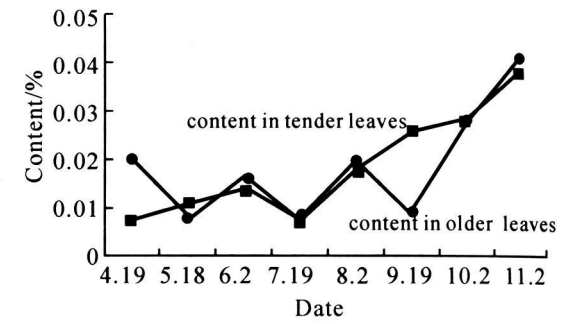

Fig. 5 Vanation of HCPT Concents in Leaves of Camptotheca Acuminata in Different Months

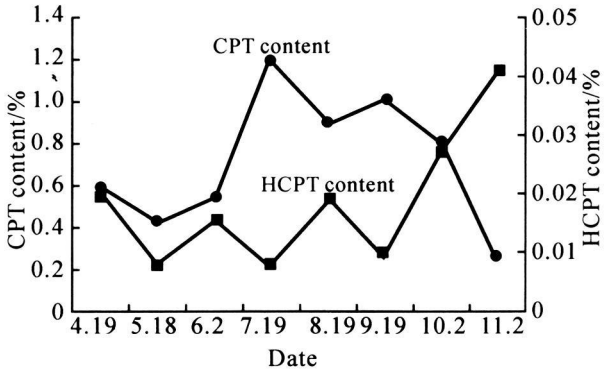

Fig. 6 Vanation of CPT and HCPT Concent in Lender Leaves of Camptotheca Acuminata in Different Months

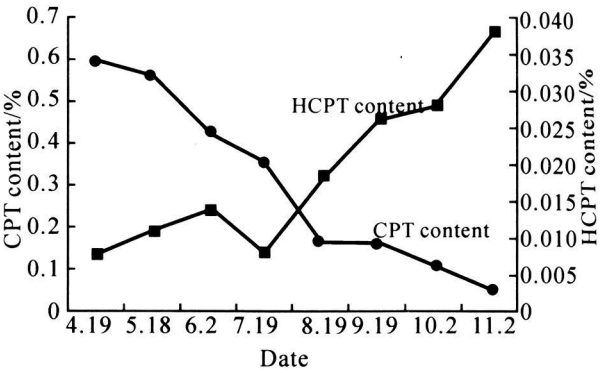

Fig. 7 Vanation of HCPT Concents in Old Leaves of Camptotheca Acuminata in Different Months

C. accuminata D. sprouted new leaves continuously from Aprial to November. Liu et al ever reported that CPT content in leaves descended eleven percent every month on the everage from April to October[21]. The results of Zhang Yu-hong et al showed that CPT content exhibited a single apex curve in young leaves from April to November and it reached the highest content in July[22]. The results of Guo Yong et al showed that the content of CPT presented two peaks curve both in tender leaves and older leaves from April to November, which Appeared in June and September respectively, It could reach the highest content in June[23].

However, the results of our test showed that, as can be seen in figure 4, in a year, the content of CPT

presented two peaks curve in tender leaves of C. acuminata, which appeared in July and September respectively and reached the highest point in July; while it declined persistently in older leaves.

The content of CPT in tender leaves was obviously higher than that in older leaves after June ($P<0.05$) which was in agreement on the whole with the theory that the CPT content in tender leaves was obviously higher than that in older leaves as a kind of chemical defence material (Liu Z et al, 1997).

As figure 5 showed, the content of 10-hydrocamptothecin in tender leaves fluctuated awfully in different months, it was quite high in April and declined notably in May, and then varied between the two levels and assented continuously after October. The 10-hydrocamptothecin content in older leaves increased persistently except for the lowest content in July. Both in tender leaves and older leaves, the content of 10-hydrocamptothecin reached the highest point in November and lowest in July.

Form figure 6 and figure 7 we can see that the accumulation of camptothecin and 10-hydrocamptothecin presented evident relativity in the different growth stages. The camptothecin and 10-hydrocamptothecin contents in tender leaves were both high in early stages. After June, the changes of the two alkaloids exhibited good mutual effects. In a year, the camptothecin accumulation decreased persistently in older leaves; while the 10-hydrocamptothecin content in older leaves increased continuously which was opposite to camptothecin except for the lowest content in July when the camptothecin acumunition in tender leaves reached the highest contents.

4 Conclusions

In this study, we established an HPLC method for the simultaneous determination of CPT and HCPT in *Camptotheca acuminata* Decne. Leaves. The method was successfully applied to analyze CPT and HCPT contents in leaves in different seasons. Our results show that the accumulation of CPT and HCPT presented evident relativity in the different growth stages. The contents of CPT and HCPT in tender leaves were both high in early stages. After June, the changes of the two alkaloids exhibited good mutual effect. The camptothecin accumulation decreased persistently in older leaves for all the growth period, while the 10-hydrocamptothecin content in older leaves was increasing continuously which was opposite to the decreasing of camptothecin accumulation. This results suggest that the change fashion between them was probably not only from camptothecin to 10-hydrocamptothecin but also from 10-hydrocamptothecin to camptothecin.

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喜树叶中喜树碱和 10- 羟基喜树碱的 HPLC 分析

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摘 要: 建立了同时测定喜树叶片中喜树碱含量的高效液相色谱分析方法. 采用 sinochrom ODS C18 分析柱(250 mm 4.6 mm BP, 5.0 m), 以甲醇/水为流动相, 检测波长为 266 nm, 梯度洗脱程序为: 在前 6 min 流动相甲醇- 水的体积比由 40% 线性增加至 50%, 并保持恒定 2 min, 在随后的 32 min 甲醇- 水的体积比线性增至 70%, 在 40 min 时停止该程序. 喜树碱在低、中、高 3 个量的平均加样回收率分别为 99.66%, 100.3%, 99.97%。10- 羟基喜树碱在低、中、高 3 个量的平均加样回收率分别为 99.87%, 99.88%, 100.4%。对不同季节的喜树嫩叶和成熟叶进行了喜树碱和 10- 羟基喜树碱含量分析, 结果表明喜树碱和 10- 羟基喜树碱的含量变化呈现明显的相关性。

关键词: 高效液相色谱法; 喜树; 叶片; 喜树碱; 10- 羟基喜树碱

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