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Original Article

Simultaneous Analysis of Topotecan and Capsaicin by Micellar Liquid Chromatography

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ABSTRACT

Objectives: This study aimed to develop a micellar liquid chromatography-based simultaneous estimation method for the analysis of topotecan and capsaicin. **Materials and Methods:** The Liquid chromatographic system consisted of the Shimadzu Prominence HPLC model (Shimadzu Corp., Kyoto, Japan) containing an LC-20AT isocratic pump, an autosampler SIL-20AC, and a diode array detector SPD-M20 A (190–800 nm) was used. The drugs were analyzed at a wavelength of 230 nm using sodium dodecyl sulfate, buffer salt (0.01 M sodium dihydrogen phosphate), and n-butanol (7%) as mobile phase. **Results:** The developed Micellar liquid chromatography-Photodiode Array Detection (MLC-PDA) method successfully eluted the topotecan and capsaicin with retention times of 6.2 min and 16.3, respectively. The developed method has displayed the limits of detection i.e. 0.05 and 0.98 µg/mL and limits of quantification i.e 0.08 and 1.25 µg/mL for topotecan and capsaicin, respectively. Statistical analysis further demonstrated that the developed method is linear, exact, accurate, and specific for the analysis of topotecan and capsaicin. The method was verified as per the ICH guidelines. **Conclusion:** The developed MLC-PDA method was found to be precise, simple, and reproducible. The developed MLC-PDA method successfully estimated the topotecan and capsaicin from the combination of drugs. Micellar chromatography is an accurate, rapid, cost-effective, and less hazardous method as compared to HPLC.

Keywords: Topotecan, Capsaicin, Simultaneous estimation, MLC-PDA, Linearity.

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INTRODUCTION

Micellar liquid chromatography (MLC) is a type of reverse phase liquid chromatography in which surfactant solutions above critical micellar concentration were used as mobile phase. Sodium dodecyl sulfate is the most widely used surfactant in micellar chromatography. MLC is a versatile technique because various interactions between solutes, micelles, and stationary phase make them suitable for both hydrophilic and hydrophobic drugs and separated the drugs in a same run.[1] The slow mass transfer from the stationary phase is the major drawback of MLC which decreases the column efficiency. The addition of a small amount of organic modifiers may solve the above problem and improve the resolution. Short-chain alcohols such as methanol, propanol, butanol, and pentanol are used as organic modifiers along with surfactant solutions. The organic modifiers enhance the efficiency and reduce the retention time to an acceptable range.[2-4] The pH of the mobile phase and temperature also play an important role in the separation of the mixture in MLC.

Topotecan is a DNA topoisomerase I inhibitor that is a water-soluble semi-synthetic derivative of camptothecin. It has demonstrated good anticancer efficacy against several solid tumors, including breast cancer ovarian cancer, and other types of cancer.[5-7] Topotecan inhibits the enzyme topoisomerase-I and prevents the religation of single-strand breaks during DNA replication. Capsaicin (trans-8 methyl-N-vanilly-6-nonenamide) is an herbal compound isolated from hot peppers. It is an alkaloidal compound used as a food additive. Capsaicin is most commonly used as an anti-inflammatory, anti-obesity, and antioxidant agent. Recently, the anticancer activity of capsaicin is also investigated for the treatment of cancer. The combination of herbal drugs with synthetic anticancer drugs displayed synergistic responses. Friedman *et al.*, (2017) reported the combination of camptothecin and capsaicin for the treatment of small cells lung carcinoma.[8] Capsaicin can modulate apoptosis and autophagy and increase the anticancer activity of camptothecin. Extensive evidence showed that capsaicin can improve the anti-cancer activity of camptothecin in treatment of solid tumor. The mechanism of a synergistic effect of camptothecin and

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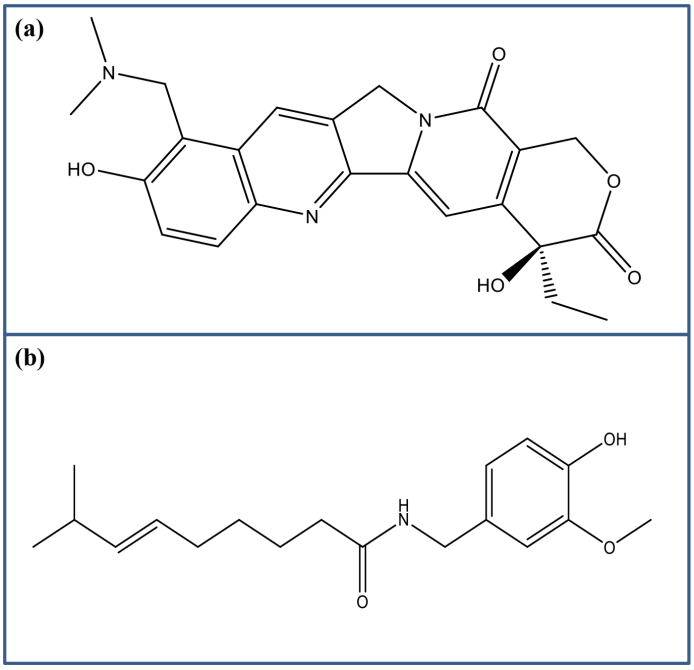

Figure 1: Chemical structure of (a) Topotecan and (b) Capsaicin.

capsaicin may be an increase in intracellular calcium and calpain pathway.[9-12] Figure 1 depicted the chemical structure of topotecan and capsaicin.

The MLC utilized the environment friendly mobile phase i.e. surfactant solution which reduced the use of hazardous organic solvents. The simultaneous estimation of topotecan and capsaicin by the MLC has not been reported in the literature but it is important to estimate both the drugs in a sample of formulation bearing combination of both drugs specially for the treatment of solid tumor. Therefore, the method for the simultaneous estimation of topotecan and capsaicin was developed using the MLC with a UV detector. The results obtained using the developed simultaneous MLC-PDA method were promising.

Materials

Topotecan was obtained as a gift sample from Fresenius Kabi Oncology Limited (Echelon Institutional Area, Gurgaon, Haryana) India. Capsaicin was purchased from Sigma-Aldrich. Sodium dodecyl sulfate, Sodium dihydrogen phosphate, n-Butanol, and HPLC grade water were purchased from Sigma-Aldrich (Powai, Mumbai, India).

Instrument

The Liquid chromatographic system consisted of the Shimadzu Prominence HPLC model (Shimadzu Corp., Kyoto, Japan) containing an LC-20AT isocratic pump, an autosampler SIL-20AC, and a diode array detector SPD-M20 A (190–800 nm) was used and the analytes were monitored at 230 nm. Chromatographic analysis was performed on Luna 5 u C-18 (2)100A column having 250 × 4.6 mm i.d. and 5 µm particle size. The column was operated at the room temperature. Chromatograms were evaluated using a computer running LC Solution software version 1.22SP1 connected to the Shimadzu Prominence HPLC instrument. Microsoft Office Excel 7 (Microsoft Corp., Seattle, WA, USA) and Origin Pro 8 (Northampton, MA, USA) were employed to process data and for several statistical calculations.

MATERIALS AND METHODS

Preparation of stock solution

The stock solution of topotecan and capsaicin (1mg/ml) was obtained by dissolving 10 mg of accurately weight topotecan and capsaicin into the 10 ml of methanol, respectively. The different aliquots of topotecan and capsaicin were prepared in the range of 0.08-10 and 1.25-20 µg/ml, respectively using the stock solution separately and methanol was used as a solvent.

Mobile phase

The micellar solution used as a mobile phase was prepared by dissolving a weighed amount of surfactant, i.e., sodium dodecyl sulfate (SDS) (0.1 M) and buffer salt [0.01 M sodium dihydrogen phosphate (NaH_2PO_4)] in deionized water using a magnetic stirrer. A small amount of organic modifier i.e. n-butanol (7%) was also added to the surfactant solution. The solution was vigorously shaken and ultrasonicated for 15 min to ensure complete solubilization. It was filtered through a 0.45 mm nylon membrane filter and stored.

Validation of the Analysis Method

The linearity, limit of detection (LOD), limits of quantification (LOQ), accuracy, precision, extraction recovery, and robustness were studied to validate the MLC method.

Linearity and Range

The calibration curves for topotecan and capsaicin were found to be linear in the range of 0.08-10 and 1.25-20 µg/ml, respectively with a correlation coefficient of 0.999 and 0.9966, respectively. The regression analysis of calibration curves is reported in Table 1. Linearity curves of topotecan and capsaicin are depicted in Figure 5 and 6, respectively.

Accuracy and precision

The accuracy and precision of the developed MLC-PDA method were analyzed using three different concentrations of drugs i.e. 0.08, 5, and 10 µg/mL of topotecan and 1.25, 5, and 10 µg/ml of capsaicin, respectively. The samples were prepared in the same solvent which was used in the calibration curve. The precision (% RSD) of the developed method was determined by measuring the intra-day and inter-day coefficient of variation and the % RSD for the repeated measurements that were statistically significant. The examination of three different drug concentrations in triplicate and three times on the same day was used to determine the intra-day precision of the chosen approach. For three consecutive days,

Table 1: Retention time and calibration data, including calibration range, linear regression, correlation coefficient (R^2), limits of detection (LODs), and quantification (LOQs) obtained for Topotecan and Capsaicin using the MLC-PDA method.

Analyte	t_R (min.)	Calibration (µg/mL) (*n*=5)						
		Range (µg/mL)	Linear regression	R^2	LODs	RSD (%)	LOQs	RSD (%)
Topotecan	6.2	0.08-10	y=1.2365x+0.058	0.9990	0.05	4.2	0.08	2.2
Capsaicin	16.3	1.25-20	y=8.102x+1.069	0.9966	0.98	6.9	1.25	5.1

Table 2: Intra-day (*n*=5) accuracy and precision for the studied compounds.

Analytes	Added Conc. (µg/mL)	Intra-day		
		Found (Mean)	Accuracy (%)	Precision RSD (%)
Topotecan	0.08	0.081	102	1.7
	5	5.05	98.1	1.9
	10	10.10	99.9	3.6
Capsaicin	1.25	1.21	99.3	3.2
	5	5.03	102	2.7
	10	10.01	103	3.6

Table 3: Inter-day accuracy and precision of the developed method.

Analytes	Added conc. (µg/mL)	Inter-day		
		Found (Mean)	Accuracy (%)	Precision RSD (%)
Topotecan	0.08	0.082	101.8	4.9
	5	5.10	104.5	4.6
	10	10.08	98.9	4.9
Capsaicin	1.25	1.22	99.6	6.2
	5	5.01	105	6.5
	10	10.10	99.2	3.9

samples were analyzed in the same manner as for the intra-day precision assay to determine the inter-day precision.

Limit of detection and limit of quantification

The lowest concentration of an analyte that can be reliably distinguished from background levels is known as the limit of detection (LOD).[13-14] The smallest amount of analyte for which a quantitative determination may be made with the necessary precision and accuracy is known as the limit of quantification (LOQ) of a specific analytical process. According to ICH recommendations, LOD and LOQ were determined using the following equation.

$$LOD = 3.3 \times \sigma / S$$

$$LOQ = 10 \times \sigma / S$$

Where σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve.

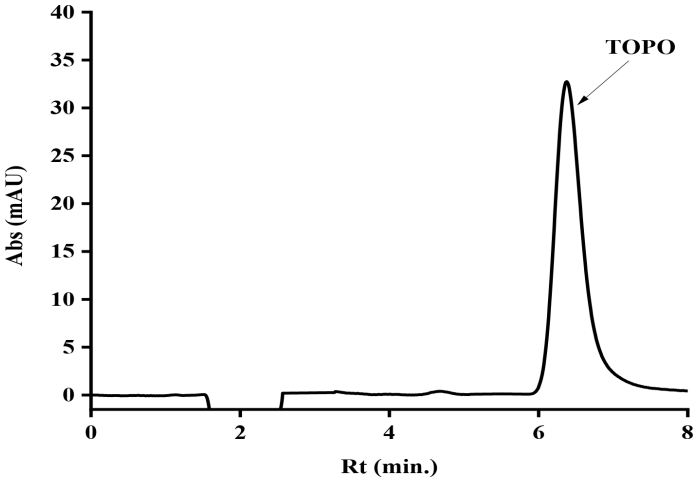

Figure 2: The Chromatogram depicted the peak of topotecan, Flow rate-1.0 ml/min, Concentration 2.5 µg/ml, Retention time: 6.2 min.

Robustness and ruggedness

The robustness and ruggedness of the method were checked by altering the chromatographic parameters like pH, flow rate, mobile phase, a wavelength of the detector, injected volume and, column temperature.

RESULTS AND DISCUSSION

The combination of topotecan and capsaicin displayed the synergistic effect for the treatment of solid tumor. The literature reported various methods for the determination of topotecan and capsaicin alone but to the best of our knowledge no method has been reported for the simultaneous analysis of topotecan and capsaicin. Therefore, simultaneous estimation method using the MLC-PDA was developed for the analysis of topotecan and capsaicin in the combination therapy. The developed MLC-PDA method was validated for analytical performance parameters such as linearity, accuracy, precision, specificity and quantification limits as per the ICH guidelines.

Chromatographic separation

The topotecan and capsaicin displayed a retention time of 6.2 min and 16.3 min, respectively. The difference in retention time of both drugs was sufficient to separate the peaks of topotecan and capsaicin. Both drugs were eluted within 17 min but 24 min run time was used to ensure the complete elution of drugs. The isocratic mode was used for elution of topotecan and capsaicin.

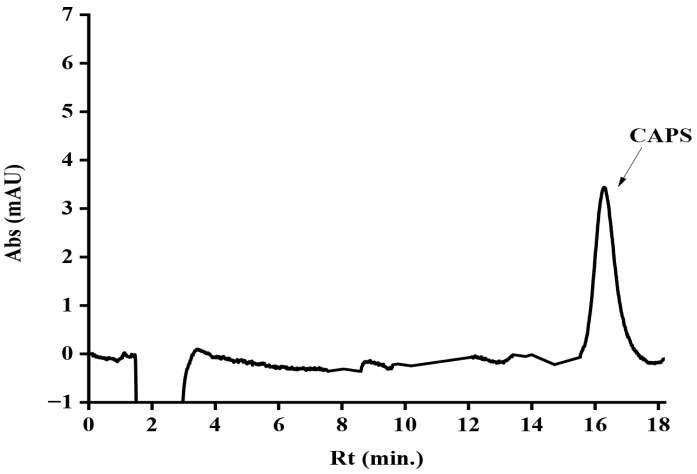

Figure 3: The Chromatogram depicted the peak of capsaicin, Flow rate-1.0 ml/min, Concentration 10 µg/ml, Retention time: 16.3 min.

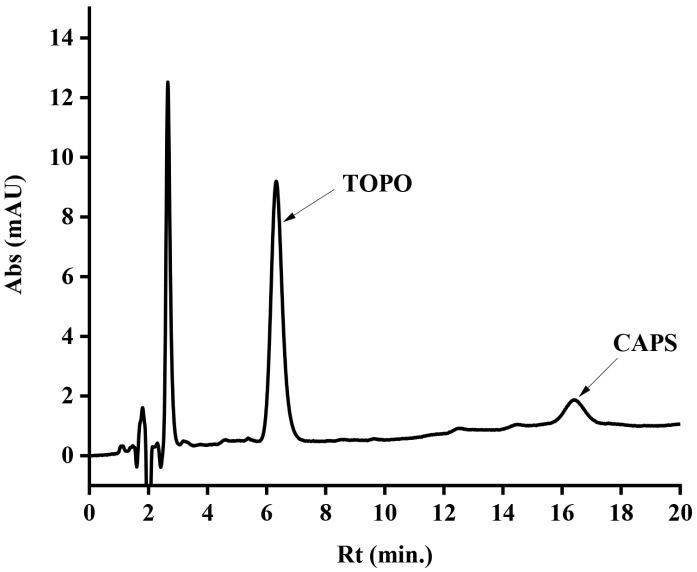

Figure 4: The Chromatogram depicted the peak of topotecan and capsaicin, Flow rate-1.0 ml/min. Retention time: 6.2 min for topotecan and 16.3 for capsaicin.

The chromatogram represented the peaks of topotecan, capsaicin and combination of topotecan and capsaicin (Figure 2, 3 and 4).

Validation of the method

The developed simultaneous estimation technique for topotecan and capsaicin was validated for various parameters such as linearity, accuracy, precision, and quantification limits according to the ICH guidelines. The chromatographic parameters of the MLC-PDA method have been summarized in Table 4 for the simultaneous estimation of both drugs.

Linearity

The linearity curve of topotecan and capsaicin were prepared in the concentration range of 0.08- 10 and 1.25-20 µg/ml, respectively. The linearity curve was plotted between concentrations of drugs (µg/ml) verse area values of the chromatogram (mAU). Table 1 displayed the linear equation and correlation coefficient of topotecan and capsaicin. The correlation coefficients of topotecan and capsaicin were found to be 0.999 and 0.9966, respectively which confirmed the linear relationship between the concentration of the drug and the area under the curve (Figure 5 and 6).

Table 4: Chromatographic parameters of MLC-PDA method employed for topotecan and capsaicin combination.

Parameter	Simultaneous estimation	
	Topotecan	Capsaicin
Wavelength	230 nm	230 nm
Linearity range	0.08-10 µg/ml	1.25-20 µg/ml
Run time	24 min	24 min
Flow rate	1 ml/min	1 ml/min
Mobile phase	0.1 M SDS, 0.01 M NaH_2PO_4	0.1 M SDS, 0.01 M NaH_2PO_4
Organic modifier	7% n-butanol	7% n-butanol
pH	7.4	7.4

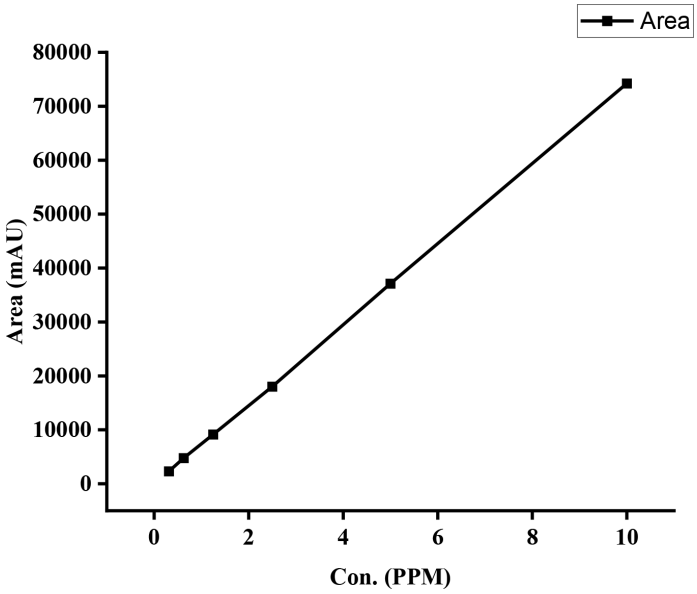

Figure 5: Graph representing the linearity curve of topotecan.

Accuracy and Precision

The accuracy of the developed method was measured by calculating the recoveries of topotecan and capsaicin by adding the known concentration of drugs in standard stock solution. The recovery of topotecan was found in a range of 98.1 to 104.5% whereas it was 99.2 to 105% for capsaicin. The values did not differ significantly after intra-day and inter-day estimation confirmed the repeatability and reproducibility of the method.

Analysis of the intra- and inter-day precision of the combination of topotecan and capsaicin standards of three different

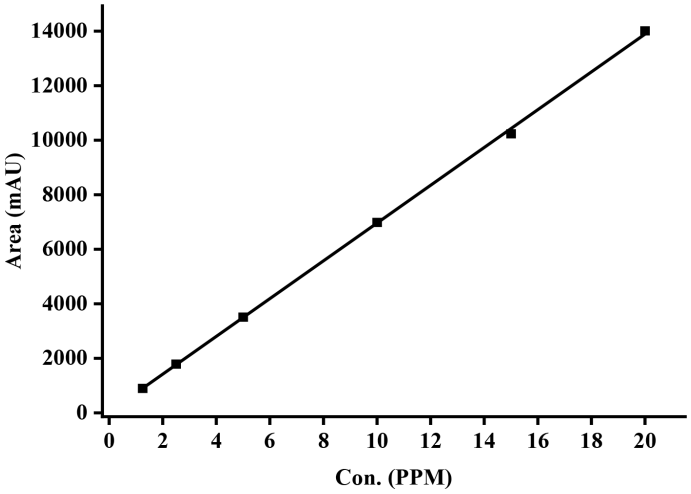

Figure 6: Graph representing the linearity curve of capsaicin.

concentrations i.e. 0.08, 5, and 10 for topotecan and 1.25, 5, and 10 for capsaicin revealed a %RSD value less than 7 % inferring an acceptable level of precision for the developed method. Table 2 and 3 shows the intra-day and inter-day accuracy and precision of the drug standards.

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were determined using the equation. The LOD of topotecan was found to be 0.05 µg/mL with 4.2% RSD while it was 0.98 µg/mL for capsaicin with 6.9% RSD. The LOQ of topotecan was found to be 0.08 µg/mL with 2.2% RSD while it was 1.25 µg/mL for capsaicin with 5.1% RSD.

Ruggedness/robustness testing

The chromatographic parameters like the flow rate, wavelength, injected volume, and the column temperature were altered to check the ruggedness/robustness of the method. Retention time, relative retention time (RRT), resolution, and plate number were the chromatographic separation characteristics that did not significantly change when the operational parameters changed.

CONCLUSION

The MLC-PDA method was developed for the simultaneous estimation of topotecan and capsaicin. The method for simultaneous estimation of topotecan and capsaicin is not reported in the literature using MLC-PDA. The developed method was found to be precise, simple, and reproducible. ICH guidelines were used for the validation of the developed method. Micellar chromatography is a simple, accurate, rapid, cost-effective, and less hazardous method as compared to HPLC. The developed MLC-PDA method successfully estimated the topotecan and capsaicin from the combination of drugs. The proposed method has displayed the limits of detection i.e. 0.05 and 0.98 µg/mL and limits of quantitation i.e 0.08 and 1.25 µg/mL for topotecan and capsaicin, respectively. For topotecan and capsaicin, the retention time was found to be 6.2 and 16.3 min, respectively, which ensures accurate measurement of both drugs in the formulation. Statistical analysis further demonstrated that the developed method is linear, exact, accurate, and specific for the analysis of topotecan and capsaicin. The developed method displayed a linear relationship with a correlation coefficient of 0.999 and 0.9966 for topotecan and capsaicin, respectively. The method's accuracy lies between 98.1% and 105.00%. The new method can therefore be used to analyze topotecan and capsaicin in pharmaceutical dosage forms regularly.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

MLC: Micellar liquid chromatography; **PDA:** Photodiode Array Detection; **SDS:** Sodium Dodecyl Sulfate; **LOD:** Limit of Detection; **LOQ:** Limits of Quantification; **ICH:** International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; **RRT:** Relative Retention Time.

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