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DETERMINATION OF SN-38 CONTENTS IN NANOCAPSULES BY CHROMATOGRAPHIC AND SPECTROPHOTOMETRIC METHODS

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ABSTRACT

A rapid and sensitive reversed phase high performance liquid chromatography (RP-HPLC) method using isocratic elution with UV detection and UV spectrophotometric method was developed for quantification of SN-38 (7-ethyl-10-hydroxycamptothecin), a camptothecin derivative in a poly (lactic-co-glycolic acid) (PLGA) based nanocapsule (PLNP). Chromatographic separation was performed on a Kromasil,KR100,C18, (4.6 mm X 250 mm, 5µm) analytical column with a mobile phase containing a mixture of octane-1-sulfonic acid sodium salt, potassium di hydrogen phosphate and acetonitrile (29:29:42v/v/v). SN-38 was detected at a wavelength of 265nm. The limit of detection (LOD) and limit of quantitation (LOQ) of HPLC method were found to be 0.006 µg mL^{-1} and 0.02 µg mL^{-1} for carboxylate and 0.002 µg mL^{-1} and 0.02 µg mL^{-1} for lactone form respectively. For UV method LOD and LOQ is 0.026 µg mL^{-1} and 0.08 µg mL^{-1} respectively. The methods were validated for linearity, accuracy, precision and robustness as per International Conference on Harmonization (ICH) guidelines and applied for determination of entrapment efficiency and drug loading in nanocapsules during *in vitro* analysis. Although HPLC method is more specific and precise and has better detection and quantification limits than UV method but quantitative determination by UV method becomes important when solvent recovery, cost effectiveness and time of analysis is important.

KEYWORDS: HPLC, UV spectroscopy, Nanocapsules, 7-ethyl-10-hydroxycamptothecin, poly (lactic-co-glycolic acid).

1. INTRODUCTION

SN-38 (7-ethyl-10-hydroxycamptothecin) is 100-1000 times more potent metabolite of Irinotecan (CPT-11)[1] and is alkaloid, camptothecin derivatives obtained from the plant *Camptotheca acuminata*. Its chemical name is (4*S*)-4,11-Diethyl-4,9-dihydroxy-1*H*-pyrano[3',4':6,7] indolizino[1,2-*b*] quinoline-3,14(4*H*,12*H*) dione. This compound cause cancer cell death by interfering with mammalian DNA topoisomerase I. CPT-11 is approved for treatment of various types of cancer like lung and colorectal cancer.[2-5] It is reported that only 2-8% of CPT-11 given to a patient is converted to SN-38. Therefore, large dose of CPT-11 is required to achieve the therapeutic effectiveness of the drug.[6] SN-38 has two forms viz. lactone and carboxylate. Lactone (more cytotoxic) form exists at acidic pH and carboxylate (less cytotoxic) form exists at basic pH. Lactone form has more tumor inhibitory activity than carboxylate form.[7-9] The poor solubility of SN-38 in any pharmaceutically acceptable solvent and its pH sensitivity limit its clinical application.[10] Several novel drug delivery systems have been developed till date, which are in different phases of clinical trials.[11] Many liquid chromatographic methods have been applied for quantification of SN-38 in human plasma,[12] simultaneous determination of lactone and carboxylate forms in rat plasma samples.[13] and total forms of irinotecan and SN-38 in human serum.[14] Most of these methods were mainly useful for therapeutic monitoring of the drug.[9-15] No visible spectrophotometric method for quantitative and qualitative determination of SN-38 in bulk drug samples and in polymeric nanocapsules was yet reported. The objective of this research is to develop and validate rapid, economical and sensitive visible spectrophotometric method for quantitative and qualitative determination of SN-38 in bulk drug samples and polymeric nanocapsules and to compare it with a RP-HPLC method. Both the methods were applied to determine the entrapment efficiency and drug content of polymeric nanocapsules of SN-38 (PLNP). The chemical structure of SN-38 and its conversion from lactone to carboxylate forms is shown in Fig.1.

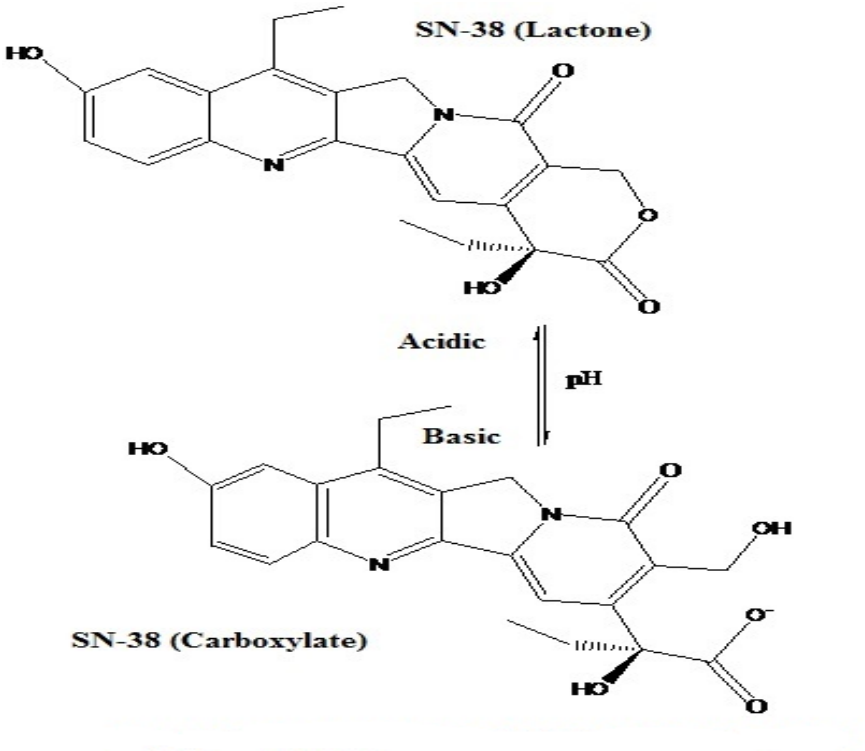


Fig 1: pH based conversion of lactone and carboxylate forms of SN-38

2. MATERIAL AND METHODS

2.1 Chemicals and reagent

SN-38 (99.8% purity) was obtained as a gift sample from Avra labs, Hyderabad, India. Poly-l-lactide co glycolide (PLGA) (50:50) was obtained as a gift sample from Evonik industries, Mumbai, India. Pluronic F-68, Acetonitrile (ACN) (HPLC grade), Octane-1-sulfonic acid sodium salt, Potassium dihydrogen phosphate (KH_2PO_4), HPLC grade water and orthophosphoric acid were purchased from Hi media, Mumbai, India.

2.2 Instrumentation

2.2.1 UV spectrophotometric system

It was performed on double beam UV-visible spectrophotometer (Shimadzu, model 1800) having two matched quartz cells with 1 cm light path with UV probe 2.34 software version. Digital pH meter model 101(Electronics India) was used for pH measurement.

2.2.2 Chromatographic system and conditions

The HPLC system consists of Young lin instrument, # 899-6 (Korea) with Vac. Degasser, Quaternary Pump, Column compartment and UV detector with software Aurochrome 3000. Column is Kromasil (C-18), pore size 100A^o, particle diameter 5 µm, with internal diameter 4.6mm and length 250mm. The mobile phase consisted of two solvents, which were 1.0 g of

Octane-1-sulfonic acid sodium salt in 500 ml of water (A) and 13.5 gm of KH_2PO_4 in 500 ml of water (B). It was mixed with acetonitrile in the ratio A: B: Acetonitrile (29:29:42 v/v/v), pH of the mixture adjusted to 3 with ortho phosphoric acid. The chromatographic behavior of SN-38 was determined by a method reported by Ebrahimnejad *et al* [16] with slight modifications. During assay, an aliquot of 20µL of diluted sample of PLNP was injected into the analytical column at 30^{o}C at a flow rate of 1 mL/min. SN-38 was detected at 265nm and quantitatively determined using an external calibration method.

2.3. Method development

2.3.1 Lactone-carboxylate forms conversion (for UV spectroscopy)

SN-38 is less soluble in acidic solutions and exists in lactone ring, which is unstable and is very soluble in basic solutions and converts to an open-ring carboxylate form (Fig 1). UV spectra was taken after scanning solutions of SN-38 at different pH as given in table 1 from 200-400nm. In acidic solutions (lactone form), the color was milky and in basic solutions (carboxylate form), it changed to yellow. The UV spectra obtained after scanning solutions between 200-400 nm at acidic pH 4.5 and at basic pH 10.5 are shown in Fig.2 and at pH 1.2, 4.5 and 7.4 are shown in Fig.3.

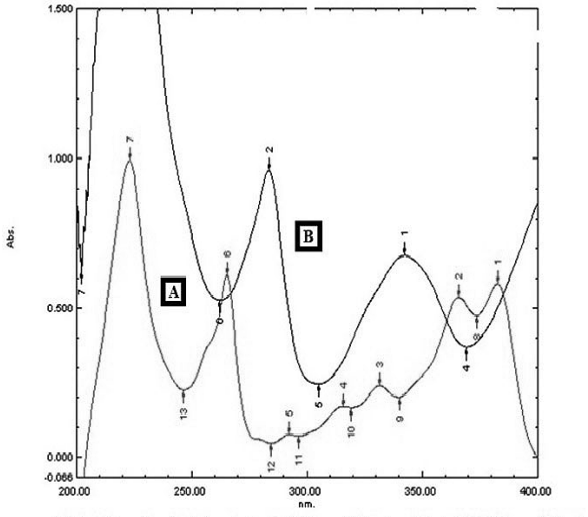


Fig 2: UV spectra of solution A: at pH 4.5 (ʎ max 265nm) and B: at pH 10.5 (ʎ max 283nm)

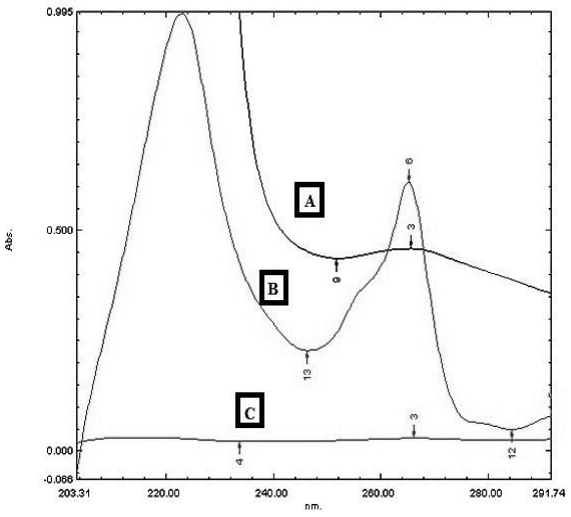


Fig 3: UV spectra of solution A: at pH 7.4 (ʎ max 265nm), B: at pH 4.5 (ʎ max 265nm) and C: at pH 1.2 (ʎ max 265nm)

Table 1: Solvents or mixture of solvents chosen for determination of λ max of SN-38 at different pH by UV spectrophotometry.

s.no	Solvents	pH	λ max (nm)
1	Acetonitrile and 0.1 M Hcl for pH adjustment (Lactone)	1.2	265 nm
2	Acetonitrile (Lactone)	4.5	265 nm
3	Acetonitrile : Water (50:50,v/v) pH adjusted by adding 0.1M NaOH (intermediate) and 1.0 M NaOH (carboxylate)	7.4 10.5	265 nm 283 nm

2.3.2 Standard stock solution for UV method

SN-38 was quite soluble in acetonitrile, (pH 4.5, 100mM). This solvent system was further used for preparing stock solutions and method validation. Accurately weighed quantity of 10 mg SN-38 reference standard was taken in 100ml volumetric flask and dissolved and diluted up to the mark with above solvent system to give a stock solution having strength 100µg/mL. Standard solutions were prepared from stock solution after proper dilution with the solvent system in the concentration range of 2-100 µg/mL. The λ max was found to be 265 nm. Calibration curve was constructed by plotting absorbance against respective concentration at 265 nm and correlation coefficient (r^2) and regression line equation was determined as shown in Fig 4.

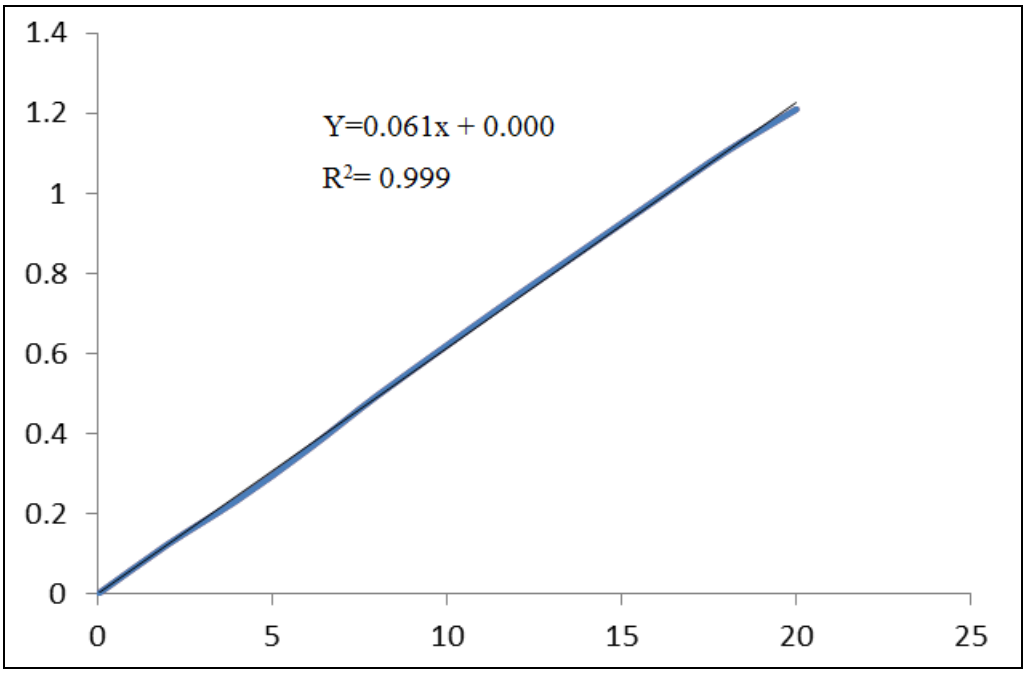
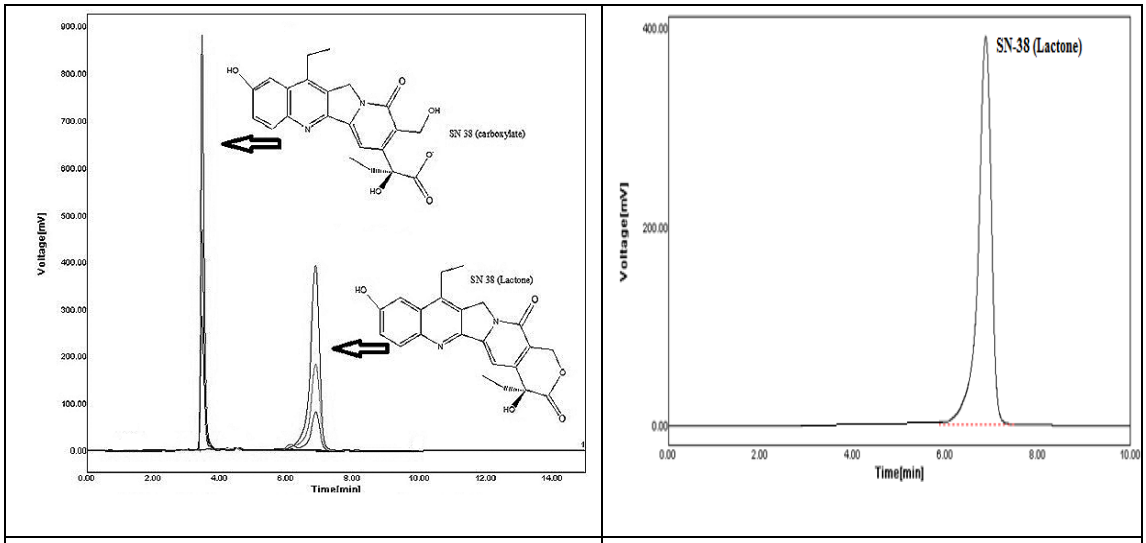


Fig 4: Calibration curve of SN-38 at 265 nm (pH 4.5) for UV spectroscopy



(A) (B)

Fig 5: (A) Overlay HPLC chromatograms of lactone and carboxylate forms of SN-38 (10, 40 and 100 µg/ml) (B) HPLC chromatogram of SN-38 content in Polymeric nanocapsule (only lactone peak was found)

2.3.3 Standard stock solution for HPLC

Standard stock solutions were prepared at 100µg/mL in acetonitrile. For lactone form, the solutions were diluted with acetonitrile pH adjusted with 0.1 M Hcl (pH 4.5, 100mM) and for carboxylate it was diluted with acetonitrile : water (50:50,v/v), pH adjusted with 0.1 M NaOH (pH 10.5, 100mM). Mobile phase was filtered with vaccum filteration assembly with 0.22µm membrane filter and ultasonicated for 10 minutes to remove air bubbles. All samples were filtered with 0.22µm membrane filter and purged with helium gas before injecting. Dilutions were taken in the range of 1-100 µg/mL. The overlay chromatograms for lactone and carboxylate forms at three different concentrations of SN-38 obtained are shown in Fig 5.

2.4 Preparation of Poly(lactide-co-glycolide) (PLGA) based polymeric nanocapsules PLNP

PLNP was prepared as reported by Prasad *et al.*[17] Briefly, PLNPs were prepared by emulsion-solvent evaporation method.[18] Pluronic F-68 solution was magnetically stirred at room temperature and then10mg of SN-38(in 1% glacial acetic acid) and 1% PLGA solution was added to it. This mixture was added slowly to the aqueous phase. It was stirred overnight then centrifuged at 2500 rpm for 10 min at 4^{o}C and supernatant sonicated for 5 minutes. It was again centrifuged and sediment washed with water and lyophilized along with 1% mannitol. It was preserved at 2-4 ^{o}C in a tightly closed container till further use.

2.5 Drug entrapment efficiency and drug loading

Drug Entrapment Efficiency (EE) was determined by ultra-centrifugation method. 2mg of PLNP was taken and dispersed in 2ml of dichloromethane. 5 ml of acetonitrile was added to the above solution and centrifuged (Remi centrifuge, Mumbai, India) at 20000 rpm for 20 min at 4^{o}C. The total drug concentrations in nanocapsules before centrifugation and in supernatant after centrifugation were determined by both UV and HPLC method at 265nm. The percentage of the drug entrapped in the PLNP was calculated as follows:

$$\text{Drug entrapment (\%)} = \frac{\text{drug total} - \text{drug supernatant}}{\text{drug total}} \times 100$$

$$\text{Drug loading efficiency (\%)} = \frac{\text{Wt of SN-38 calculated}}{\text{Wt of nanocapsules}} \times 100$$

2.6 Stability of drug Solution

Stability of the drug solution was determined based on the (%) recovery of SN-38 solution at 10 µg /mL and PLNP at room temperature (around 25°C) for four consecutive days, samples taken 3 times daily, quantified by both UV and HPLC methods for lactone and carboxylate forms.

2.7 Method Validation

Both methods (UV and HPLC) were validated for accuracy, precision, linearity, limit of detection, limit of quantitation and robustness as per ICH guidelines.[18]

2.7.1 Linearity and range

2.7.1.1 UV spectroscopy

Linearity was found after analyzing the solutions at different concentration range between 2-100 µg /mL. In the concentration range of 2-20 µg /mL, the absorbance of SN-38 was less than 1.0 and calibration curve was linear. It was determined by analyzing six independent levels of calibration curve in the range of 2-20µg /mL. Absorbance of each solution against blank solvent system was recorded at 265 nm. Curve of absorbance vs. concentration was plotted and correlation co-efficient and regression line equation for SN-38 were determined.

2.7.1.2 HPLC

Linearity was established by analyzing ten concentrations of lactone and carboxylate forms ranging between 10-100 µg/mL by plotting the peak area against the corresponding concentration. UV detection was at 265 nm. SN-38 standards at five different concentrations

with a minimum of 90–110% of the expected concentration of SN-38 in PLNP were included in the study. The calibration graphs were validated by the high value of the correlation coefficient (>0.99) as given in Fig 6.

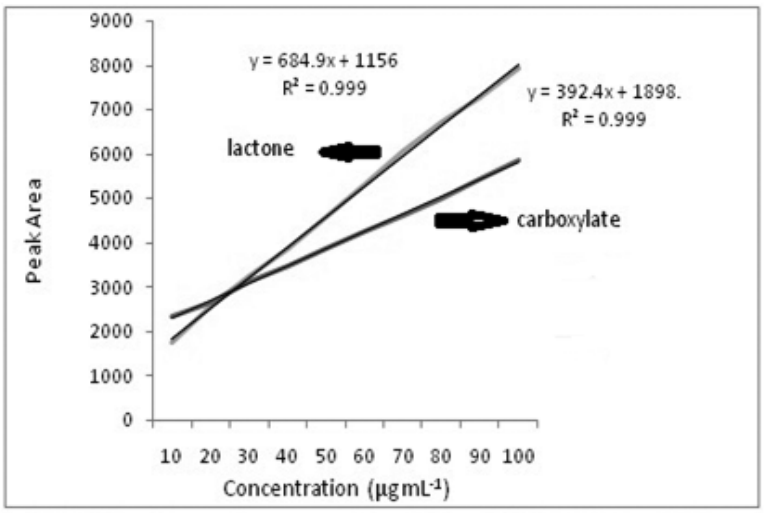


Fig 6: Calibration curve of lactone and carboxylate forms of SN-38 at 265 nm for HPLC chromatograms

2.7.2 Precision (for UV and HPLC)

The precision was evaluated by repeatability (intra-day) and intermediate precision (inter-day). The repeatability was done by analyzing three aliquots of PLNP sample from the same container and the same SN-38 standards. The precision was measured by the %R.S.D. of the triplicate assays for each of the three samples. The same process was repeated on a second day to assess intermediate precision using three freshly prepared aliquots of PLNP. %RSD was calculated. Similarly for HPLC, precision is evaluated as mentioned above for both lactone and carboxylate forms.

2.7.3 Accuracy (for UV and HPLC)

Accuracy was determined by performing recovery studies by adding different concentrations of SN-38 to blank polymeric nanocapsules at three different levels of 80%, 100% and 120% of the expected concentration of SN-38 in PLNP at three different times. The amount of SN-38 was calculated at each level and % recoveries were computed. Same process was repeated for HPLC for both lactone and carboxylate forms.

2.7.4 Robustness

Robustness for UV method was determined by analyzing the SN-38 sample solution (10µg /mL) by different analysts on different days and at different temperatures (25^{o}C, 30^{o} C and 35^{o} C) and was repeated six times. Robustness for HPLC method was performed by deliberately changing the chromatographic conditions. The flow rate of the mobile phase was changed from 1.0 mL min^{-1} to 0.9 mL min^{-1} and 1.1 mL min^{-1} while ratio of the mobile phase was changed by ± 1%.

2.7.5 Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were estimated from the set of six calibration curves used to determine method linearity.

LOD= 3.3*σ/S and LOQ= 10*σ/S

Where, σ = the standard deviation of y-intercepts of regression lines

S = the slope of the calibration curve

2.7.6 Analysis of entrapment efficiency (EE) and drug loading of SN-38 in PLNP

Both UV and HPLC methods were applied to determine drug Entrapment Efficiency (EE) in nanocapsules. For entrapment efficiency of SN- 38 by HPLC, the quantity of SN-38 in PLNPs was calculated by the following formula: 100C (r_t/ r_s) in which C is the concentration of SN-38 reference standard in standard solution (in µg /mL); and r_t and r_s are the SN-38 peak responses (Peak area) of test and standard solution, respectively. For analysis by UV, the quantity of SN-38 in PLNPs is calculated after suitable dilutions with acetonitrile and then calculating the concentration with the external calibration curve.

2.7.7 Data analysis

. All statistical calculations were performed with Microsoft office excel 2007 and Graph pad prism 6 (trial version). Results were considered statistically significant if calculated P values were 0.05 or less.

3. RESULTS

3.1 Lactone and carboxylate forms

SN-38 underwent structural changes from acidic pH to basic pH which was depicted by the UV spectra taken at different pH. The changes in the absorbances at different pH clearly showed the transformation from lactone to carboxylate form. Lactone forms were found at 265 nm at pH 1.2, 4.5, and 7.4 and at pH 10.5, only carboxylate form was present which

showed its characteristic peak at 283 nm. Similarly for HPLC, the chromatogram for lactone form was depicted at 6.87 minutes and the chromatogram for carboxylate form was depicted at 3.45 minutes. This pH-dependent structural conversion was found to be reversible.

3.2 Method Validation

3.2.1 UV method

Linearity was observed in the concentration range of 2-20 µg/mL with correlation co-efficient 0.999.Accuracy of the method was confirmed by recovery study from formulation at three level of standard addition. % Recovery for SN-38 was found to be 99.93 -100.3 with Percent of Relative Standard Deviation (% RSD) between 0.1-0.18. % as shown in table 2. Intra-day and inter-day precision is shown in table 3. Intra-day precision was in the range of % R.S.D (0.08-0.14) and inter-day precision was in the range of % R.S.D (0.04-0.13). Robustness test results are shown in table 4.

Limit of detection is the lowest concentration that can be detected and limit of quantification is the lowest concentration which can be quantified. LOD and LOQ were found to be 0.026 µg/mL and 0.08µg/mL respectively. Summary of validation parameters for both the methods are given in table 6. Stability studies result is given in table 8. It shows that pure SN-38 solution in lactone form gets degraded by nearly 25% at room temperature after 4 days while PLNP is degraded by 9% after 4 days.

3.2.2 HPLC method

Linearity was observed in the concentration range of 10-100 µg /mL with correlation coefficient 0.999.The average percent recoveries of SN-38 in the spiked samples at three different levels ranged from 99.98 to 100.12 % for carboxylate and 100.03-100.13 % for lactone form. The %R.S.D. for the triplicate measurements at each level was less than 0.5% (Table 2).Intra-day and inter-day precision is shown in table 3. Intra-day precision was in the range of % R.S.D (0.07-0.19) and inter-day precision was in the range of % R.S.D (0.05-0.14) for carboxylate form, while for lactone form, intra-day precision was in the range of % R.S.D (0.05-0.18) and inter-day precision was in the range of % R.S.D (0.06-0.31),

Robustness test results are shown in table 5. LOD and LOQ were found to be 0.006 µg /mL and 0.02 µg /mL for carboxylate form and 0.002 µg /mL and 0.02 µg /mL for lactone form respectively. Stability studies of lactone and carboxylate forms at room temperature for 4

days showed that lactone forms were degraded by 22%, carboxylate forms were degraded by 18 % whereas PLNP's were degraded by 9%.

Table 2: Results of accuracy study

UV spectrophotometry			HPLC			
			Carboxylate		lactone	
Level of recovery	Recovery (%)	% RSD	Recovery(%)	% RSD	Recovery (%)	% RSD
SN-38 added at 80% level	100.15	0.18	100.04	0.06	100.03	0.21
SN-38 added at 100% level	100.30	0.10	100.12	0.08	100.13	0.21
SN-38 added at 120% level	99.93	0.15	99.98	0.07	100.07	0.15

Table 3: Results of precision for UV and HPLC

S.No	Sample	UV spectrophotometry		HPLC			
				carboxylate		lactone	
		Intraday	Interday	Intraday	Interday	Intraday	Interday
		% RSD (triple assays)	% RSD (triple assays)	% RSD (triple assays)	% RSD (triple assays)	% RSD (triple assays)	% RSD (triple assays)
1	Assay-1	0.08	0.04	0.16	0.09	0.13	0.06
2	Assay-2	0.14	0.13	0.19	0.14	0.18	0.12
3	Assay-3	0.13	0.06	0.07	0.05	0.05	0.31
	Overall % RSD (9 samples)	0.85	1.12	0.71	0.88	0.72	0.83

Table 4: Data for robustness test for UV spectrophotometry

S.No	Variable parameters	Assay Results (%)[a]
1	Analyst 1 Analyst 2	101.2 ± 0.08 100.1 ± 0.04
2	Day 1 Day 2	101.4 ± 0.11 101.2 ± 0.12
3	25°C (for 24 hrs) 28°C (for 24 hrs) 30°C (for 24 hrs)	99.98 ± 0.02 99.94 ± 0.04 98.01 ± 0.06

[a]Values indicate mean ± S.D where n=6

Table 5: Influence of changes in experimental parameters on the performance of chromatographic system

Parameter	Modification	Retention time (minute)[a]		Peak area (%change)[a]		Tailing Factor[a]	
		Carboxylate	Lactone	Carboxylate	Lactone	Carboxylate	Lactone
Mobile phase ratio (v/v/v) A:B:Acetonitrile	28:30:42	3.46	6.87	0.11	0.12	1.42	0.71
	30:28:42	3.45	6.82	n/a	0.08	1.41	0.70
	30:30:40	3.47	6.85	0.15	0.09	1.42	0.70
Flow rate (ml/min)	1.0	3.45	6.87	0.45	n/a	1.42	0.71
	0.9	3.48	6.89	n/a	0.36	1.42	0.71

	1.1	3.39	6.79	0.08	0.43	1.41	0.70
Temperature (◦C)	30	3.45	6.87	0.03	n/a	1.41	0.71
	28	3.45	6.87	n/a	0.05	1.42	0.70
	32	3.45	6.87	0.13	n/a	1.42	0.71

[a] The results were reported as an average of five replicate injections.

Table 6: Summary of validation parameters of UV spectroscopy and HPLC method

S.No.	Parameters		Results (UV)	Results (HPLC)	
				Carboxylate	Lactone
1	λ max		265 nm	265 nm	265 nm
2	Regression line equation		Y= 0.061x + 0.000	Y=392.4x+ 1898	Y=684.9x +1156
3	Correlation coefficient (r^2)		0.999	0.999	0.999
4	Precision (% RSD)	Intra Day	0.08-0.14	0.07-0.19	0.05-0.18
		Inter day	0.04-0.13	0.05-0.14	0.06-0.31
5	Accuracy (% Recovery)		99.93-100.3	99.98-100.12	100.03-100.13
6	LOD[a]		0.026 µg/ml	0.006 µg/ml	0.002 µg/ml
7	LOQ[b]		0.08 µg/ml	0.02 µg/ml	0.02 µg/ml
[a]LOD-Limit of detection, [b]LOQ- Limit of quantitation					

3.3 Method Application

Both methods were applied to study drug loading and SN-38 entrapment efficiency by ultra centrifugation method. The entrapment efficiency was more than 72% and drug loading more than 9.9% of PLNP 5 (Table 7) and no carboxylate peak was detected in SN-38 loaded PLNP's chromatogram (Fig.5). The stability studies showed that after 4 days study the SN-38 content was more than 91% in lactone form. It was also confirmed that no other components of PLNP interfered with SN-38 determination.

Table 7: Entrapment efficiency and drug loading of SN-38 in PLNPs

Optimized Nanocapsules	Entrapment efficiency (%)		Drug loading (%)	
	HPLC	UV	HPLC	UV
PLNP 4	71.48 ± 1.31	71.51 ± 1.36	9.1 ± 0.31	9.21± 0.45
PLNP 5	72.58 ± 0.95	72.6 ± 1.02	9.99 ± 0.26	10.00 ± 0.47
PLNP 6	72.09 ± 0.82	72.1 ± 0.98	8.63 ± 0.41	8.67± 0.12

Table 8: Stability studies of SN-38 solution and PLNP during 4 consecutive days at 10µg mL^{-1}.

Days	HPLC						UV			
	Lactone		Carboxylate		PLNP		SN-38 solution (Lactone form)		PLNP	
	% recovery[a]	% RSD	% recovery[a]	% RSD	% recovery[a]	% RSD	%recovery[a]	% RSD	% recovery[a]	% RSD
1	99.06 ± 0.77	0.78	98.77 ± 0.99	1.01	99.03 ± 0.95	0.96	99.2 ± 0.63	0.64	99.42 ± 0.81	0.81
2	88.72 ± 1.08	1.22	92.45 ± 0.52	0.57	95.38 ± 0.53	0.55	88.69 ±1.01	1.13	95.29 ± 0.46	0.48
3	83.38 ± 0.53	0.63	88.72 ± 1.08	1.22	92.96 ± 0.95	1.02	83.21 ±0.79	0.94	92.86 ± 0.73	0.79
4	77.72 ± 1.08	1.39	81.72 ± 1.08	1.33	91.11 ± 0.75	0.82	76.52 ±0.95	1.2	91.69 ± 1.21	1.48

[a]Values indicate mean ± S.D where n=3

4. DISCUSSIONS

An optimized reversed phase HPLC and UV methods are proposed for effective determination of lactone and carboxylate forms of SN-38 in bulk form and in polymeric nanocapsule carriers. This study provides a simple procedure with better chromatographic peak resolution in lesser run time that prevents potential degradation due to long analysis time. In accuracy study, the % R.S.D. for the triplicate measurements at each level was less than 1.0%, which demonstrated a high level of accuracy. The % R.S.D. values for absolute peak areas of SN-38 in samples were less than 1.0% for both intra-day precision and inter-day precision. These data demonstrated acceptable precision of the method. LOD and LOQ results depicts that the method is amply suitable for determination of SN-38 in pharmaceutical formulations even in very low concentration. Robustness studies shows that the method is quite robust and the variability in the mobile phase ratio, flow rate and slight differences in temperature do not incur significant changes in the results. By and large, the method confirmed sufficient robustness and appropriateness for the analysis of SN-38 nanocapsules under the deliberately altered HPLC conditions. Similarly, UV method demonstrated a high level of accuracy, precision and robustness with lactone form detected at 265 nm. Handling by different analysts on different days under slight differences in temperature do not incur any significant changes in the results. It can be used for detection of carboxylate forms at 283nm as well (data not shown). The overall % RSD for both intra-day

precision and inter-day precision is less than 1.0% with overall % RSD less than 1.12%. These data also demonstrated acceptable precision of the method.

5. CONCLUSIONS

Both UV and HPLC methods for the determination of SN-38 was found to be precise, accurate and selective. Even though SN-38 was formulated in a complex polymeric nanocapsule, present methods occupied no sample pre-treatment or extraction procedures. RP-HPLC with UV detector can determine both lactone and carboxylate forms simultaneously by using ion separating agents with very low LOD and LOQ and less time of retention. Whereas when only lactone or carboxylate form is to be analyzed, direct UV method can be used as it is more rapid and economical. The purpose of the new spectrophotometric method is not to replace the available advance methods for the analysis of SN-38, but to serve as an alternative method to be used where advanced instruments e.g. HPLC are not available for routine analysis. In addition, it avoids costly columns without wastage of solvents. Comparing the results of entrapment efficiency and drug loading determined by both the processes imparted no significant difference at 95% significance level. Statistical data analysis confirmed that there is no significant change with a change in different variable parameters. Both methods were found to be suitable and robust for analysis of SN-38 in PLNPs in preclinical studies and for routine analysis of SN-38 in pharmaceutical preparations.

6. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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