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Validated UHPLC-DAD method for quantification of cholesteryl-succinyl-5-fluorouracil conjugate

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The aim of the present study was to develop and validate an UHPLC-DAD method for quantification of cholesteryl-succinyl-5-fluorouracil conjugate in a standard sample, a lipid nanoemulsion and dissolved samples. The separation of the conjugate was carried out on Hypersil GOLD 50 X 2.1 mm RP C_{18} column packed with 1.9 µm packing as a stationary phase. The mobile phase was methanol:water (80:20 % v/v) at a flow rate of 0.3 ml/min with DAD detection at 276 nm. The proposed method was found to be precise, accurate, robust, sensitive and specific for quantification of the conjugate. The utility of the proposed method was checked by the assay of conjugate in a lipid nanoemulsion and in dissolved samples. High assay value of conjugate in the lipid nanoemulsion was observed (99.25 %). *In vitro* dissolution of the cholesteryl-succinyl-5-fluorouracil conjugate in the lipid nanoemulsion was observed as 78.1% after 24 h. The conjugate was found to be sufficiently degraded under acid, base and thermal stress conditions. The developed method successfully resolved the drug conjugate peak in the presence of its degradation products. These results indicated that the developed UHPLC-DAD method can successfully be used for routine analysis of drug conjugates in pharmaceutical formulations.

Keywords: UHPLC-DAD, Cholesteryl-succinyl-5-fluorouracil, Dissolution, Lipid nanoemulsion, Validation.

INTRODUCTION

The 5-fluorouracil (5-FU) is a well known antitumor drug which belongs to the antimetabolite class of anticancer drugs and is recommended clinically for the treatment of various types of tumors such as colorectal, breast and ovarian tumor either alone or in combination with other antitumor drugs [1, 2]. Nevertheless, due to incomplete and erratic bioavailability profile upon oral administration, it has been administered *via* intravenous injection whereby the drug plasma concentration could not be maintained for long time because of rapid metabolism of drug [3, 4]. In the last few decades, the prodrug/conjugation approach has been successfully applied for enhancing therapeutic efficacy and reducing adverse effects associated with 5-FU [5-7]. Therefore, in the present study, the cholesteryl ester conjugate of 5-FU (cholesteryl-succinyl-5-fluorouracil with molecular weight of 598.38, Figure 1) was synthesized in order to improve its therapeutic efficacy of 5-FU and to reduce oral/parenteral adverse effects.

For quantification of 5-FU, various analytical methods such as gas chromatography, capillary electrophoresis, spectrophotometry, colorimetry and high performance liquid chromatography (HPLC) have been reported [8-21]. Ultra high performance liquid chromatography (UHPLC) is a relatively newly developed liquid chromatographic technique which offered several advantages over routine HPLC method such as high sensitivity, high analytical speed, improved resolution and short analysis run time [22-24]. Nevertheless, no UHPLC methods have been reported for the quantification of 5-FU or any of its conjugate/derivative or prodrug in literature so far. Therefore, in the present study, attempts were made to develop and validate a rapid, facile, precise, accurate, robust and stable UHPLC method coupled with diode array detector (DAD) for rapid analysis of the newly synthesized conjugate cholesteryl-succinyl-5-fluorouracil conjugate utilizing isocratic elution, taking into consideration the test conditions recommended by the International conference on harmonization (ICH) [25]. The developed method is completely novel for the cholesteryl-succinyl-5-fluorouracil conjugate. The developed method was applied for the assay of cholesteryl-succinyl-5-fluorouracil conjugate in a lipid nanoemulsion and

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in vitro dissolved samples of the lipid nanoemulsion.

EXPERIMENTAL

Chemicals and reagents

5-FU was donated by Alfa Aesar (Ward Hill, MA). Cholesteryl-succinyl-5-fluorouracil conjugate (Mol. Wt. 598.38, Figure 1) was synthesized and characterized in the laboratory. HPLC grade methanol, hydrochloric acid (HCl), sodium hydroxide (NaOH) and hydrogen peroxide (H_2O_2) were purchased from BDH Laboratory supplies (Liverpool, UK).

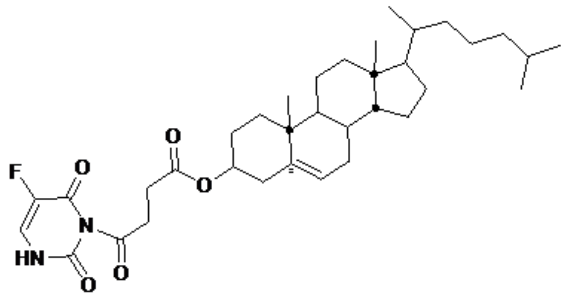

Figure 1 Molecular structure of cholesteryl-succinyl-5-fluorouracil conjugate (mol. wt. 598.38)

Cholesterol, cholesteryloleate, phosphatidylcholine and glycerol trioleate (triolein) were purchased from Sigma Aldrich (St. Louis, MO). Ultra-pure water was obtained from a ELGA water purification system (Wycombe, Bucks, UK). All other chemicals and reagents were of analytical reagent grade.

Instrumentation and chromatographic conditions

Chromatographic identification of the cholesteryl-succinyl-5-fluorouracil conjugate was performed at room temperature (22±1°C), with a Thermo Scientific UHPLC system (Thermo Scientific, Germany) equipped with a 3000 LC pump, 3000 autosampler, binary pumps, programmable DAD detector, Ultimate 3000 column oven, Ultimate 3000 controller and an online vacuum degasser. Chromeleon software (version 6.8) was used for data analysis. Chromatographic identification of the conjugate was performed on a Thermo Hypersil GOLD 50 × 2.1 mm RP C_{18} column (Thermo Scientific, Germany) with a 1.9 µm packing as a stationary phase. The mobile phase consisted of methanol: water (80:20 % v/v). The elution was performed at a flow rate of 0.3 ml/min with diode array detection (DAD) detection at 276 nm. Samples (1 µl) were injected using an Ultimate 3000 series Thermo auto sampler.

Preparation of cholesteryl-succinyl-5-fluorouracil conjugate stock solution

The calibration curve for the cholesteryl-succinyl-5-fluorouracil conjugate was plotted in the concentration range of 1 to 50 µg/ml. Stock solution of 100 µg/ml was prepared. Serial dilutions were made from the stock solution by diluting the required aliquots with the mobile phase to get concentrations in the range of 1 to 50 µg/ml.

Method development

Various solvent systems were checked as mobile phase for the development of a suitable UHPLC-DAD method for the quantification of cholesteryl-succinyl-5-fluorouracil conjugate in its standard drug compound. The selection of mobile phase was mainly based on assay sensitivity, retention time, peak parameters, suitability for stability studies, ease of preparation and cost effectiveness of the solvents. Based on these criteria, several mobile phases such as acetonitrile-water, acetonitrile–phosphate buffer, methanol–water, methanol–phosphate buffer, ethanol–water and ethanol-phosphate buffer at different proportions were used. Finally, the combination of methanol–water (80:20 % v/v) was selected as an eluent for the further studies.

Validation studies

The proposed UHPLC-DAD method was validated for various parameters such as linearity, accuracy, precision, sensitivity, robustness and specificity according to ICH guidelines [25].

Freshly prepared linearity solutions in the concentration range of 1-50 µg/ml were used for construction of the calibration curves. The mobile phase consisting of methanol–water (80:20 % v/v) was delivered at a rate of 0.3 ml/min for column equilibration; the baseline was continuously monitored during this process. The chromatographic detection was performed at 276 nm. The prepared dilutions were injected in triplicate; peak areas were recorded using the UHPLC system for each dilution and concentration was plotted against peak area.

The accuracy of the proposed method was assessed by a previously reported standard addition method [26]. The standard cholesteryl-succinyl-5-fluorouracil conjugate solution (10 µg/ml) was spiked with 0, 50, 100 and 150 % of extra standard drug solution and was reanalyzed by the proposed method. Percent recovery (%), percent relative

standard deviation (%RSD) and standard error for each concentration were calculated.

The precision of the proposed UHPLC-DAD method was determined as repeatability (intraday precision) and interday (intermediate precision). Intraday precision of the proposed method was measured by quantification of four different concentrations of cholesteryl-succinyl-5-fluorouracil conjugate (10, 15, 20 and 25 μg/ml) in triplicate on the same day. Intermediate precision was measured by repeating the studies on three different days.

Limit of detection (LOD) and limit of quantification (LOQ) of the proposed method were determined by the signal to noise ratio (S/N ratio) method as reported previously using the following equations:

$LOD = 3.3 \times S/N$ and $LOQ = 10 \times S/N$

The robustness of the proposed method was determined to evaluate the effect of deliberate variation of chromatographic conditions on the determination of the cholesteryl-succinyl-5-fluorouracil conjugate. A target concentration of 10 μg/ml of the conjugate was selected for this purpose. The robustness of the proposed method was determined by changing the mobile phase flow rate from 0.3 ml/min to 0.35 and 0.25 ml/min; the wavelength of detection from 276 to 280 and 272 nm and the concentration of methanol in the mobile phase from 80 to 85 and 75 %.

Forced degradation studies

Forced degradation studies were performed under various stress conditions such as acid stress, base stress, oxidative stress and thermal stress. These studies were performed to evaluate the stability and specificity of the proposed UHPLC-DAD method.

For acid- and base-induced degradation, the target concentration (10 μg/ml) of cholesteryl-succinyl-5-fluorouracil conjugate was freshly prepared in the mobile phase. An aliquot (1 ml) of this solution was exposed to acid and base hydrolysis by adding 4 ml of 0.1M HCl or 4 ml of 0.1M NaOH, respectively. Acid and base treated mixtures were kept in a hot-air oven for 48 h at 60°C and then analyzed by the proposed method for determination of cholesteryl-succinyl-5-fluorouracil conjugate in the presence of its acid and base degradation products, respectively.

The same procedure was adopted for oxidative degradation studies using 3% H_2O_2 as an oxidant.

For thermal degradation, an aliquot of the target concentration of cholesteryl-succinyl-5-fluorouracil conjugate (10 μg/ml) was exposed to a hot-air oven for 48 h at 60°C and then analyzed by the proposed method for determination of cholesteryl-succinyl-5-fluorouracil conjugate in the presence of its thermal degradation products.

Preparation and characterization of a lipid nanoemulsion

A lipid nanoemulsion of cholesteryl-succinyl-5-fluorouracil conjugate was prepared according to the method reported by Moura and coworkers [27]. Briefly, the lipid phase was emulsified with the aqueous phase (deionized water) by prolonged ultrasonic irradiation followed by two-step ultracentrifugation with density adjustment by addition of KBr to obtain a lipid nanoemulsion [28]. The composition of the lipid phase was as follows: 40 mg of cholesteryloleate, 20 mg of phosphatidylcholine, 1 mg of triolein and 0.5 mg of cholesterol. 6 mg of cholesteryl-succinyl-5-fluorouracil conjugate was solubilized in ethanol and introduced into the lipid nanoemulsion. The prepared lipid nanoemulsion was then sonicated, dialyzed and filtered as reported previously [27, 28].

The lipid nanoemulsion of cholesteryl-succinyl-5-fluorouracil conjugate was characterized for droplet size, PI, viscosity and RI. The droplet size and PI were determined using Malvern Mastersizer (Malvern Instruments Ltd., Worcestershire, UK) at room temperature (25°C) at a scattering angle of 90°. The procedure for the measurement of droplet size and PI was similar to that reported in our previous article [29]. Viscosity and RI of the cholesteryl-succinyl-5-fluorouracil loaded lipid nanoemulsion were determined using Brookfield viscometer (Brookfield Engineering Laboratories, Inc, Middleboro, MA) and Abbes type refractometer (Precision Standard Testing Equipment Corporation, Germany), respectively, at 25±1°C as reported in ref. [29].

Assay of cholesteryl-succinyl-5-fluorouracil conjugate in the lipid nanoemulsion

The utility of the proposed UHPLC-DAD method was checked by applying this method for the quantification of cholesteryl-succinyl-5-fluorouracil conjugate in the lipid nanoemulsion. For determination of the cholesteryl-succinyl-5-fluorouracil conjugate content in the lipid nanoemulsion (containing 6 mg/ml of cholesteryl-succinyl-5-fluorouracil conjugate), 1 ml of the formulation was suitably diluted with mobile phase (methanol:water-80:20) to obtain 100 ml of stock solution [30]. The stock solution was sonicated for about 1 h and subjected to UHPLC analysis for

determination of the cholesteryl-succinyl-5-fluorouracil conjugate content after suitable dilution with mobile phase. The possible interactions between the components of the lipid nanoemulsion and the cholesteryl-succinyl-5-fluorouracil conjugate were also studied.

Dissolution studies of the lipid nanoemulsion

The utility of the proposed method was also checked by applying the method in dissolution/drug release studies of cholesteryl-succinyl-5-fluorouracil conjugate from the lipid nanoemulsion. Dissolution studies were performed in 500 ml of deionized water using the United States Pharmacopoeia (USP) XXIV method at a speed of 100 rpm at 37±0.5°C [28]. One ml of the formulation was placed in a dialysis bag (MWCO 12,000 g/mol; Spectrum Medical Industries, Mumbai, India). Samples (1 ml) were withdrawn at different intervals of time (0, 1, 2, 3, 6, 18 and 24 h) and were replaced by the same amount of fresh deionized water. The samples were analyzed for the cholesteryl-succinyl-5-fluorouracil conjugate content by the proposed method.

RESULTS AND DISCUSSION

Method development

During method development step, he use of ethanol-water and ethanol-phosphate buffer as the mobile phases resulted in an asymmetric peak with a high tailing. Further, acetonitrile-water and acetonitrile-phosphate buffer were tried at different proportions at a flow rate of 0.3 ml/min. These also resulted in a very poor peak with high tailing. Further, methanol-water and methanol-phosphate buffer were tried as mobile phases. Finally, the proportions of methanol and water were adjusted to obtain a rapid and simple assay method for cholesteryl-succinyl-5-fluorouracil conjugate with a reasonable run time, suitable retention time and acceptable tailing or asymmetry factor. Of several compositions of methanol and water and methanol and phosphate buffer investigated, the binary proportion at 80:20 % v/v was found yield a sharp peak with suitable retention time and good asymmetry. Finally, the proportions of methanol and water were adjusted to obtain a rapid and simple assay method for cholesteryl-succinyl-5-fluorouracil conjugate with a reasonable run time, suitable retention time (0.60±0.001 min) and acceptable tailing or asymmetry factor (Figure 2a).

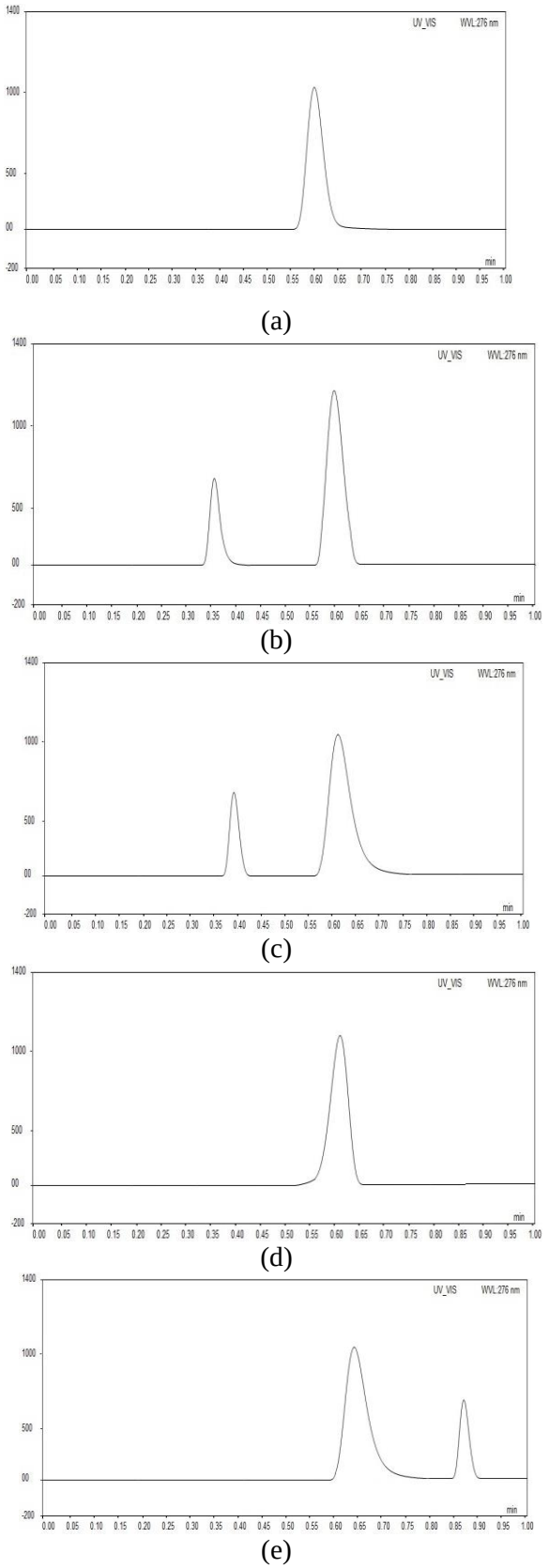

Fig. 2. UHPLC-DAD chromatogram of cholesteryl-succinyl-5-fluorouracil conjugate (a) and chromatograms in the presence of 0.1M HCl (b), 0.1M NaOH (c), 3% H_2O_2 (d) and under thermal condition (e)

Method validation

The calibration curve was plotted as a dependence of UHPLC peak areas on concentration. The data were evaluated by linear least square analysis. The calibration curve was found to be linear in the concentration range of 1–50 μg/ml**.** The regression equation was Y = 0.021 X+0.041 with a correlation coefficient (R^2) of 0.999±0.001 (Table 1).

Table 1. Linear regression data for the calibration curve of cholesteryl-succinyl-5-fluorouracil conjugate (*n*=3)

Parameters	Values
Linearity range	1– 50 μg/ml
Correlation coefficient (R^2 ± SD)	0.999± 0.001
Regression equation	Y= 0.021X+0.041
Slope ± SD	0.021 ± 0.001
Confidence interval of slope*	0.018-0.023
Standard error of slope	0.00057
Intercept ± SD	0.041 ± 0.003
Confidence interval of intercept*	0.033-0.048
Standard error of intercept	0.00173

* 95% confidence interval

No significant difference was observed in the slopes/intercepts of calibration curves (ANOVA, $p > 0.05$). The linear regression data for calibration curve of cholesteryl-succinyl-5-fluorouracil conjugate are listed in Table I.

The accuracy of the proposed UHPLC-DAD method was determined as % recovery and the results are listed in Table 2.

Good recoveries (98.12–99.60 %) of the spiked cholesteryl-succinyl-5-fluorouracil conjugate were obtained with lower values of %RSD and standard errors at each concentration level. The high recoveries pointed to the good accuracy of the developed UHPLC-DAD method.

The results of intra-day and intermediate precision were expressed in terms of % RSD and are listed in Table 3.

The results indicated that the proposed UHPLC-DAD method was precise as the % RSD values for intraday and intermediate precision were in the range of 1.51-1.84 and 1.43-1.94, respectively. Moreover, the low values of % RSD indicated the good precision of the proposed UHPLC-DAD method.

The LOD and LOQ for the proposed UHPLC-DAD method were determined by the S/N ratio method and were found to be 0.50 and 1.50 μg/ml, respectively. The low values of LOD and LOQ indicated the good sensitivity of the proposed method.

For robustness, the SD, % RSD and standard error of the peak areas for all parameters (mobile phase composition, wavelength of detection and flow rate) at a concentration level of 10 μg/ml were determined. The results are shown in Table 4.

The low values of % RSD and standard error obtained after introducing small deliberate changes in the mobile phase composition, wavelength of detection and flow rate indicated the robustness of the proposed method.

Forced degradation studies

Forced degradation studies were performed to evaluate the stability and the specificity of the proposed method. Forced degradation of cholesteryl-succinyl-5-fluorouracil conjugate was determined by exposing a target concentration under various stress conditions. The results of the forced degradation studies are listed in Table 5 and Figures 2b-e.

Table 2. Accuracy of the UHPLC-DAD method (% recovery, n = 3)

% of conjugate added to analyte	Theoretical concentration (μg/ml)	Measured concentration (μg/ml) ± SD	RSD (%)	Standard error	% Recovery
0	10	9.86±0.16	1.62	0.09	98.60
50	15	14.73±0.21	1.42	0.12	98.20
100	20	19.92±0.28	1.40	0.16	99.60
150	25	24.53±0.34	1.38	0.19	98.12

Table 3. Precision of UHPLC-DAD method (n = 3)

Concentration (μg/ml)	Repeatability (Intra-day precision)			Intermediate precision (Inter-day)		
	Mean area ± SD	RSD (%)	Standard error	Mean area ± SD	RSD (%)	Standard error
10	0.2614 ± 0.0044	1.68	0.0025	0.2702 ± 0.0047	1.73	0.0027
15	0.3856 ± 0.0071	1.84	0.0040	0.3907 ± 0.0076	1.94	0.0043
20	0.4808 ± 0.0082	1.70	0.0047	0.4771 ± 0.0072	1.50	0.0041
25	0.5819 ± 0.0088	1.51	0.0050	0.5789 ± 0.0083	1.43	0.0047

Table 4. Robustness of the UHPLC-DAD method (n = 3)

Parameters	Mean area ± SD	RSD (%)	Standard error	Retention time ± SD	RSD (%)	Standard error
Mobile phase composition						
(85:15 % v/v)	0.2788±0.0048	1.72	0.0027	0.586 ± 0.004	0.68	0.0023
(75:25 % v/v)	0.2594±0.0039	1.50	0.0022	0.632 ± 0.007	1.10	0.0040
Mobile phase flow rate						
(0.35 ml/min.)	0.2509±0.0034	1.35	0.0019	0.575 ± 0.005	0.86	0.0028
(0.25 ml/min.)	0.2745±0.0046	1.67	0.0026	0.674 ±0.008	1.86	0.0046
Detection wavelength (nm)						
272	0.2642±0.0033	1.24	0.0019	0.604 ±0.005	0.82	0.0028

Table 5. Results of forced degradation studies (n = 3)

Stress condition	Mean area ± SD	RSD (%)	Standard error	Number of degradation products (R_t)	5-FU conjugate remaining (μg/ml)	Amount recovered (%)
0.1M HCl	0.1787±0.0031	1.73	0.0017	1 (0.362)	6.55	65.57
0.1M NaOH	0.1573±0.0028	1.78	0.0016	1 (0.390)	5.53	55.38
3% H_2O_2	0.2452±0.0042	1.71	0.0024	-	9.72	97.23
Thermal	0.2104±0.0037	1.75	0.0021	1 (0.893)	8.06	80.66

65.57% of the cholesteryl-succinyl-5-fluorouracil conjugate was found to remain in the acid induced sample and 34.43% was degraded (Figure 2b). The acid degradation product (peak 1 in Figure 2b) was found to be eluted with a retention time of 0.362 min (Table V). It was also found to be degraded sufficiently in the presence of 0.1M NaOH solution (alkaline condition). 55.38% of cholesteryl-succinyl-5-fluorouracil conjugate was remaining in the alkaline stress sample and 44.62% was degraded within 48 h (Figure 2c). The base-induced degradation product (peak 1 in Figure 2c) was found to be eluted with a retention time of 0.390 min. However, 97.23% of cholesteryl-succinyl-5-fluorouracil conjugate remained in the H_2O_2 induced sample and only 2.77% degraded (Figure 2d). Therefore, cholesteryl-succinyl-5-fluorouracil conjugate was found to be sufficiently stable under oxidative stress conditions. On the other hand, 19.34% of cholesteryl-succinyl-5-fluorouracil conjugate was found to be degraded under thermal conditions and 80.66% was remaining in the solution after 48 h (Figure 2e). The thermal-induced degradation product (peak 2 in Figure 2e) was found to be eluted with a retention time of 0.893 min. Therefore, cholesteryl-succinyl-5-fluorouracil conjugate was found to be stable under oxidative stress conditions while it degraded sufficiently under acid, base and thermal stress conditions. Generally, the forced degradation studies indicated that the proposed method was specific and stable.

Characterization of the lipid nanoemulsion

Lipid nanoemulsion formulation of cholesteryl-succinyl-5-fluorouracil conjugate was successfully prepared and characterized in terms of droplet size, polydispersity index (PI), viscosity and refractive index (RI). The results of the characterization studies are listed in Table 6.

Table 6. Physicochemical characterization of cholesteryl-succiny-5-fluorouracil loaded lipid nanoemulsion

Parameters	**Value**
Droplet size (nm)[a]	71.210±5.870
PI	0.287
Viscosity (cp)[a]	152.130±8.210
RI[a]	1.343±0.002

Polydispersity index (PI); Refractive index (RI); mean ± SD, n=3

The mean droplet size of the lipid nanoemulsion was 71.21±5.87 nm with a low value of PI (0.287) which indicated the proper development of lipid nanoemulsion. However, the viscosity of the lipid nanoemulsion was found to be 152.13±8.21 cp (Table VI). The RI of the lipid nanoemulsion was 1.343±0.002 which is close to the RI of water (1.33). The lower values of droplet size, PI, viscosity and RI supported the proper development of the lipid nanoemulsion formulation of cholesteryl-succinyl-5-fluorouracil conjugate.

Assay of cholesteryl-succinyl-5-fluorouracil conjugate in the lipid nanoemulsion

According to the validation studies, the proposed UHPLC-DAD method is rapid, precise, accurate and sensitive for the quantification of the cholesteryl-succinyl-5-fluorouracil conjugate. Therefore, the developed UHPL-DAD method was applied for the quantification of cholesteryl-succinyl-5-fluorouracil conjugate in a lipid nanoemulsion. The assay value of cholesteryl-succinyl-5-fluorouracil conjugate in the lipid

nanoemulsion was found to be 99.25±1.97 %. This assay value was found to be highly acceptable according to the regulatory guidelines of ICH. The % RSD in the assay of cholesteryl-succinyl-5-fluorouracil conjugate was 1.98% which was also within the limits. High assay value and low % RSD of cholesteryl-succinyl-5-fluorouracil conjugate in the lipid nanoemulsion indicated that the proposed UHPLC-DAD method could be applied for routine analysis of derivatives/conjugates/prodrugs of 5-FU in lipid nanoemulsions and other formulations. No interactions between cholesteryl-succinyl-5-fluorouracil and nanoemulsion excipients were observed. The UHPLC-DAD chromatogram of cholesteryl-succinyl-5-fluorouracil conjugate extracted from the lipid nanoemulsion was found to be the same as that of standard cholesteryl-succinyl-5-fluorouracil conjugate, indicating the purity of the peak in the lipid nanoemulsion.

Dissolution studies of the lipid nanoemulsion

In vitro drug release (dissolution) profile of cholesteryl-succinyl-5-fluorouracil conjugate from the lipid nanoemulsion was also determined by the proposed method. The results of the *in vitro* drug release (dissolution) profile of cholesteryl-succinyl-5-fluorouracil conjugate from the lipid nanoemulsion are presented in Figure 3. About 68% of cholesteryl-succinyl-5-fluorouracil conjugate was found to be released from the lipid nanoemulsion after 6 h, as shown in Figure 3.

Initially, the release profile was of the rapid/immediate type. After a period of 6 h, the lipid nanoemulsion showed a sustained release profile of cholesteryl-succinyl-5-fluorouracil conjugate. The % dissolution of cholesteryl-succinyl-5-fluorouracil conjugate after 24 h was found to be 78.1%. Drug release studies showed that the proposed UHPLC-DAD method could also be applied for dissolution/release studies of pharmaceutical dosage forms containing 5-FU derivatives as the active ingredients.

CONCLUSION

The proposed UHPLC-DAD method for quantification of cholesteryl-succinyl-5-fluorouracil conjugate is simple, accurate, precise, robust, sensitive and stable. High assay value of cholesteryl-succinyl-5-fluorouracil conjugate was obtained in a developed lipid nanoemulsion. The drug release profile of cholesteryl-succinyl-5-fluorouracil conjugate from lipid nanoemulsion was within the acceptable limits. The method was found to be economic and efficient. These factors make the proposed method superior for routine analysis of cholesteryl-succinyl-5-fluorouracil conjugate in standard drugs, pharmaceutical formulations and dissolution samples. Because of its stability, the proposed UHPLC-DAD method can be utilized for the prediction of half life and shelf life of cholesteryl-succinyl-5-fluorouracil conjugate in pharmaceutical formulations.

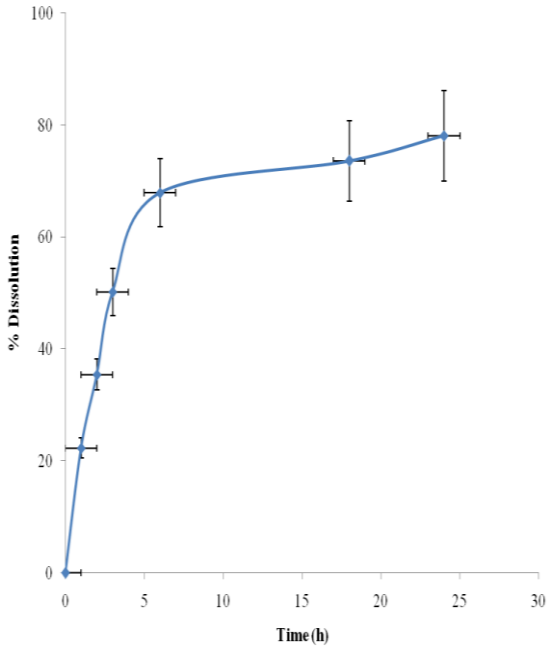

Fig. 3. *In vitro* dissolution (drug release) profile of cholesteryl-succinyl-5-fluorouracil conjugate from the lipid nanoemulsion through dialysis membrane

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CONFLICT OF INTEREST

The authors report no conflict of interest related with this manuscript.

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ВАЛИДИРАН UHPLC-DAD МЕТОД ЗА КОЛИЧЕСТВЕНО ОПРЕДЕЛЯНЕ НА ХОЛЕСТЕРИЛ-СУКЦИНИЛ-5-ФЛУОРОАЦИЛОВИ КОНЮГАТИ

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(Резюме)

Целта на настоящата работа е да се разработи и валидира UHPLC-DAD метод за количествено определяне на холестерил-сукцинил-5-флуороацилови конюгати в стандартни проби, наноемулсии и разтворени проби. Разделянето на конюгатите е извършвано на колона Hypersil GOLD 50 X 2.1 mm RP C_{18} с размери на частиците 1.9 µm като неподвижна фаза. Подвижната фаза е смес от метанол и вода (80:20 % об.) при дебит 0.3 мл/мин. С детектор DAD при 276 nm. Установено е че предложеният метод е прецизен, точен, стабилен, чувствителен и специфичен за количественото определяне на конюгатите. Приложимостта на предложения метод е изпитана при анализа на конюгати в липидни наноемулсии и в разтворени проби. Установени са високи съдържания на конюгати в наноемулсиите (99.25 %). Наблюдавано е i*n vitro* разтваряне на холестерил-сукцинил-5-флуороациловите конюгати до 78.1% след 24 часа. Установено е, че конюгатите се разтварят достатъчно при стресови въздействия с киселина, основа или повишена температура. Разработеният метод успешно определя пика на лекарствения конюгат в присъствие на неговите разпадни продукти. Тези резултати показват, че разработеният UHPLC-DAD мерод може да бъде използван успешно за рутинни анализи на конюгати на лекарства във фармацевтични препарати.