Asian Journal of Chemistry Vol. 22, No. 5 (2010), 3966-3972

Simultaneous Determination of Irinotecan Hydrochloride and its Related Compounds by High Performance Liquid Chromatography Using Ultraviolet Detection

ALI MOHAMMADI*†, FARNAZ ESMAEILI‡, RASSOUL DINARVAND‡, FATEMEH ATYABI¶ and RODERICK B. WALKER§

Department of Drug and Food Control, Faculty of Pharmacy, Tehran University of Medical Sciences, P.O. Box: 14155-6451, Tehran-14174, Iran
Fax: (98)(216)461178; Tel: (98)(216)482607; E-mail: alimohammadi@tums.ac.ir

A new simple, precise and accurate high performance liquid chromatography (HPLC) method was developed and validated for the simultaneous determination of irinotecan (CPT-11) and two related compounds *viz*., 7-ethyl-10-hydroxycamptothecin (SN-38) and camptothecin (CPT) in pharmaceutical dosage forms. Chromatography was accomplished using a reversed-phase C_{18} column and ultraviolet (UV) detection and an isocratic mobile phase consisting of 3 % v/v triethylammonium acetate buffer (pH 3) and acetonitrile (70:30 v/v). The linear range of quantitation for all the compounds was 0.1-10 µg/mL. The limit of quantitation for all the compounds ranged between 0.01-0.05 µg/mL. The method has the requisite accuracy, selectivity, sensitivity and precision to assay of CPT-11 and related compounds in pharmaceutical dosage forms and bulk API.

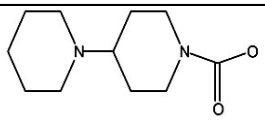
Key Words: Irinotecan, Camptothecin, High performance liquid chromatography-ultra violet, 7-Ethyl-10-hydroxycamptothecin.

INTRODUCTION

Camptothecin (CPT) is a natural, water-insoluble quinoline alkaloid produced by the plant *Camptotheca acuminata* that is indigenous to China. Camptothecin has been chemically modified to produce other anticancer agents including irinotecan (CPT-11)[1]. Irinotecan is essentially a prodrug and is hydrolyzed by carboxylesterases to form the active metabolite, 7-ethyl-10-hydroxycamptothecin (SN-38)[2]. These compounds exit as two coexisting forms *viz*., the active lactone form and an inactive carboxylate form which has no topoisomerase I inhibitive activity. The lactone form

†Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran.
‡Nanotechnology Research Centre, Tehran University of Medical Sciences, Tehran, PO Box 14155-6451, Iran.
¶Department of Pharmaceutics, Faculty of Pharmacy, Novel Drug Delivery Systems Laboratory, Tehran University of Medical Sciences, Tehran, P.O. Box: 14155-6451, Tehran-14174, Iran.
§Department of Pharmaceutics, Faculty of Pharmacy, Rhodes University, Grahamstown 6140, South Africa.

has a closed α-hydroxy-δ-lactone ring, which can be hydrolyzed to form an open-ring hydroxylic acid (carboxylate form) (Fig. 1). Under acidic conditions (pH < 5) the lactone structure predominates, whereas at pH values above 8 the carboxylate is present exclusively[3]. High-performance liquid chromatography coupled with fluorometric[1-11] detection and mass spectrometry[12,13] have been used for the quantitation of these compounds as the intact lactone-carboxylate or as a total form in buffered solutions and in biological matrices including plasma, red blood cells, saliva, urine, faeces, microsomes and in tumor tissues. A review of these methods when focusing on CPT-11 and its major metabolite, SN-38 as compounds of interest reveal important difference including the matrices from which they are isolated, separation mode *i.e.*, isocratic or gradient elution, buffer type, sample preparation, stationary phase, additives and/or ion pairing agents. The *in vitro* analysis of CPT-11 in IV solutions is necessary to ensure that products of suitable quality are manufactured and made available to the public. Furthermore *in vitro* analytical methods for CPT-11 are vital for the evaluation of new formulations of the CPT-11. Such methods may be necessary for determining encapsulation efficacy or the *in vitro* release characteristics of particulate delivery systems of CPT-11. Therefore there is a need for a simple analytical method for the determination of CPT-11 and its related compounds during product development studies. In this study we report the development and validation of a simple isocratic HPLC method for the determination of the lactone form of the CPT-11 and its main related compounds (SN-38 and CPT) in commercial products using UV detection.

Compound	R1	R2
CPT-11		C_2H_5
CPT	H	H
SN-38	OH	C_2H_5

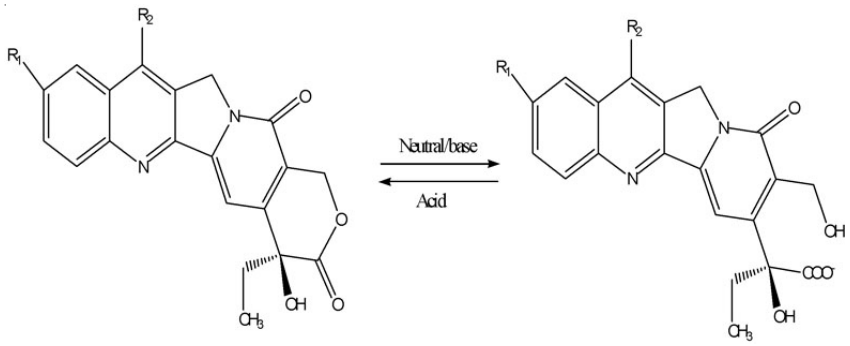


Fig. 1. Structure of the lactone and carboxylate forms of CPT-11, CPT and SN-38

EXPERIMENTAL

Irinotecan (CPT-11) hydrochloride trihydrate, 7-ethyl-10-hydroxycamptothecin (SN-38) and camptothecin (CPT) were purchased from Aurisco Pharmaceutical (Shanghai, China). HPLC-grade acetonitrile was purchased from Merck (Darmstadt, Germany). Triethylammounium acetate (TEAA) buffer was purchased from Fluka (Switzerland). Citric acid and boric acid were purchased from Merck (Darmstadt, Germany). Camptosar® injectable solutions containing 100 mg/5 mL CPT-11 HCl was obtained from the Sobhan Chemotherapeutics Company (Rasht, Iran). Water was purified using a Milli-Q® UV plus System (Millipore, Bedford, MA, USA). All other reagents were at least of analytical reagent grade and used without further purification.

Chromatographic analyses were performed using an Agilent® HPLC system (Agilent Technologies, USA) that was comprised of a degasser (Model G1322A), quaternary pump (Model G1311A), autosampler system (Model G1329A) and a diode-array detector (Model G1315B). Chemstation® software (Rev.B.02.01-SR1 [260]) was used for data monitoring and analysis. The sample injection volume was 20 µL and separation of the compounds of interest was achieved using a Nucleosil® RP-18 (5 µm, 250 mm × 4 mm) analytical column protected by a C_{18} Nucleosil® guard column (5 µm, 4 mm × 4 mm) and a mobile phase consisting of 3 % v/v triethylammonium acetate (TEAA) buffer (pH 3) and acetonitrile (70:30 v/v) at a flow rate of 1 mL min^{-1}. All samples and the column compartment were maintained at an ambient temperature of 25 °C and the eluant was monitored using UV detection at 254 nm. The mobile phase was filtered through a 0.45 µm Chrom. Tech. Nylon-66 filter prior to use.

Preparation of stock and standard solutions: Stock solutions (100 µg/mL) of CPT-11, SN-38 and CPT were prepared in ethanol (99.9 % v/v). Standard solutions (0.1, 1, 2, 5 and 10 µg/mL) of each of the compounds were prepared by pipetting appropriate aliquots of the relevant stock solution and making up to volume with the TEAA buffer solution.

Preparation of injections for analysis: Samples of commercial product were prepared for analysis by emptying the contents of 10 Camptosar® vials into a volumetric flask after which the solution was mixed well. An aliquot of the resultant solution equivalent to the volume of one injection was added into each of six 100 mL. A grade volumetric flasks made up to the volume with the TEAA buffer solution and mixed for a further 2 min. A 50 microlitre aliquot of the dilute solution was transferred to a 10 mL A grade volumetric flask and made up to the volume with the TEAA buffer solution to yield an approximate concentration of CPT-11 of 5 µg/mL which falls in the previously described range of linearity for this method.

RESULTS AND DISCUSSION

HPLC method development and optimization: The retention times for CPT-11, SN-38 and CPT using the previously described chromatographic conditions were 5.7 ± 0.14, 11.9 ± 0.12 and 14.7 ± 0.10 min (n = 40), respectively. Representative

chromatograms for CPT-11, SN-38 and CPT are shown in Fig. 2. The retention of all compounds was dependant on the strength of the buffer used to prepare the mobile phase and the pH of that buffer. In an attempt to control peak tailing, due to interaction between the analytes and underivatized silanols on the column wall, triethylammonium acetate was used. At pH values above 3.5 silanols act as weak acids react strongly with basic compounds, thereby delaying elution of the peaks of interest and resulting in peak broadening and tailing.

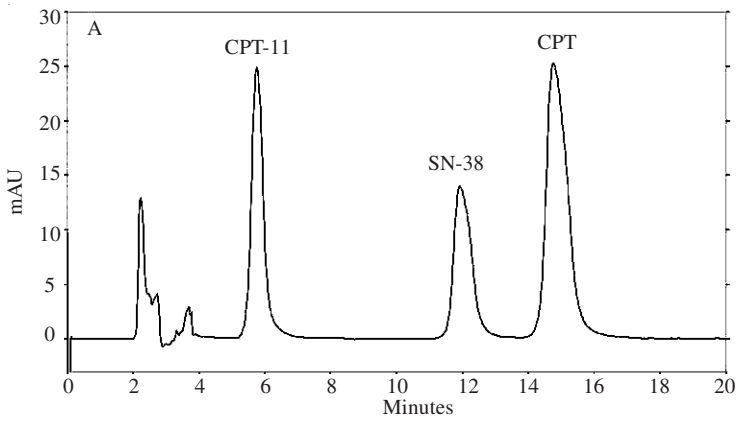


Fig. 2. A representative chromatogram of a standard solution containing CPT-11, SN-38 and CPT

Validation: The method was validated with respect to the followng parameters including linearity, limit of quantitation and limits of detection (LOQ and LOD), precision, accuracy, selectivity and recovery.

Linearity: Peak area of the compounds of interest were plotted *versus* concentration and least squares linear regression analysis performed on the resultant curve. The calibration curves constructed for CPT-11, SN-38 and CPT were found to be linear over the concentration range of 0.1-10 µg/mL with mean correlation coefficients (r) > 0.999 with % RSD values ranging from 0.2 to 3.5 % obtained following linear regression analysis.

LOQ and LOD: LOQ was determined based on signal-to-noise ratios and were selected using an analytical response of 10 times the background noise[14]. The LOQ was found to be 10, 50 and 25 ng/mL for CPT-11, SN-38 and CPT, respectively. The LOD was determined based on signal-to-noise ratios and was selected based on analytical responses of 3 times the background noise. The LOD was found to be 4, 15 and 9 ng/mL for CPT-11, SN-38 and CPT, respectively.

Precision: The precision of the assay was investigated with respect to both repeatability and reproducibility. Repeatability was assessed by injecting replicate samples (n = 9) of each of the 0.1, 1 and 10 µg/mL standards. Inter-day precision was assessed by injecting the same standards (n = 3) on three consecutive days. Within- and inter-day precision data are summarized in Table-1.

TABLE-1
PRECISION AND ACCURACY OF THE HPLC METHOD IN MEASURING KNOWN CONCENTRATIONS OF CPT-11, SN-38 AND CPT

Compound	Target concentration ($\mu g\ mL^{-1}$)	With within-day (n = 6)			Between-day (n = 6)		
		Observed concentration (mean ± SD)	RSD (%)	Accuracy (%)	Observed concentration (mean ± SD)	RSD (%)	Accuracy (%)
CPT-11	0.1	0.098 ± 0.003	3.06	98.00	0.102 ± 0.003	2.94	102.00
	1.0	1.023 ± 0.01	0.97	102.30	0.985 ± 0.012	1.22	98.50
	10.0	9.82 ± 0.15	1.53	98.20	9.790 ± 0.05	0.51	97.90
SN-38	0.1	0.101 ± 0.002	1.98	101.10	0.1015 ± 0.0025	2.46	101.50
	1.0	1.016 ± 0.02	1.96	101.60	1.022 ± 0.021	2.05	102.20
	10.0	10.02 ± 0.10	1.00	100.20	10.0 ± 0.03	0.30	100.00
CPT	0.1	0.103 ± 0.003	2.90	103.00	0.10± 0.0025	2.50	100.00
	1.0	0.997 ± 0.002	0.23	97.00	1.03 ± 0.015	1.45	103.00
	10.0	10.17 ± 0.22	2.16	101.72	10.25 ± 0.035	0.34	102.50

Accuracy: The accuracy of the assay was assessed by interpolation of replicate peak areas of three accuracy standards (0.1, 1 and 10 μg/mL) of each compound from a calibration curve prepared as previously described. In each case, the per cent relative error was calculated and is summarized in Table-1. Accuracy was determined as the per cent error by which the mean concentration of the replicate samples deviated from the known target concentration as described below: (mean concentration/ target concentration × 100). The accuracy of the assay ranged from 97-103 % at the concentrations assessed for all analytes (Table-1).

Selectivity: Typical chromatograms developed following the analysis of CPT-11, SN-38 and CPT are shown in Fig. 2 and clearly reveal the selectivity of the method for separation of irinotecan and its related compounds. Photodiode array detection was used to provide additional evidence of the selectivity of the method, and to evaluate the homogeneity of the each of the peaks of interest. Chromatographic peak purity data was obtained from the spectral analysis report and peak purity values of greater than 0.999 indicated the presence of an homogenous peak for the compounds under investigation.

Recovery: Spiked samples of CPT-11, SN-38 and CPT were prepared by addition of standard solutions to placebo injections at different percentages of the expected concentration of each compound in dilute injection solutions. Each vial of Camptosar® injection (100 mg/5 mL) contains 225 mg sorbitol NF powder and 4.5 mg of lactic acid (USP-grade) at a pH of 3.0-3.8[15]. Mean recoveries were found to be above 99.65 % at low concentration and above 99.85 % at high concentrations (Table-2).

Assay: The proposed method was applied to the determination of CPT-11 in Camptosar® injections. A typical chromatogram obtained following the assay of Camptosar® injections is depicted in Fig. 3. The results of this analysis yielded 102.4 % (% RSD = 1.12 %) of label claim indicating that the method is suitable for the selective analysis of CPT-11 and is free from interference of excipients used in this dosage form.

TABLE-2
MEAN RECOVERY OF CPT-11, SN-3 AND CPT AT LOW (n = 6)
AND HIGH (n = 6) CONCENTRATIONS OF THE ANALYTES

Analyte	CPT-11	SN-38	CPT
Low Concentration			
Concentration (μg mL^{-1})	0.1	0.1	0.1
Mean recovery (%)	99.65	101.13	98.71
RSD (%)	1.25	2.70	3.01
High Concentration			
Concentration (μg mL^{-1})	10	10	10
Mean recovery (%)	100.18	101.27	99.55
RSD (%)	0.45	1.25	1.05

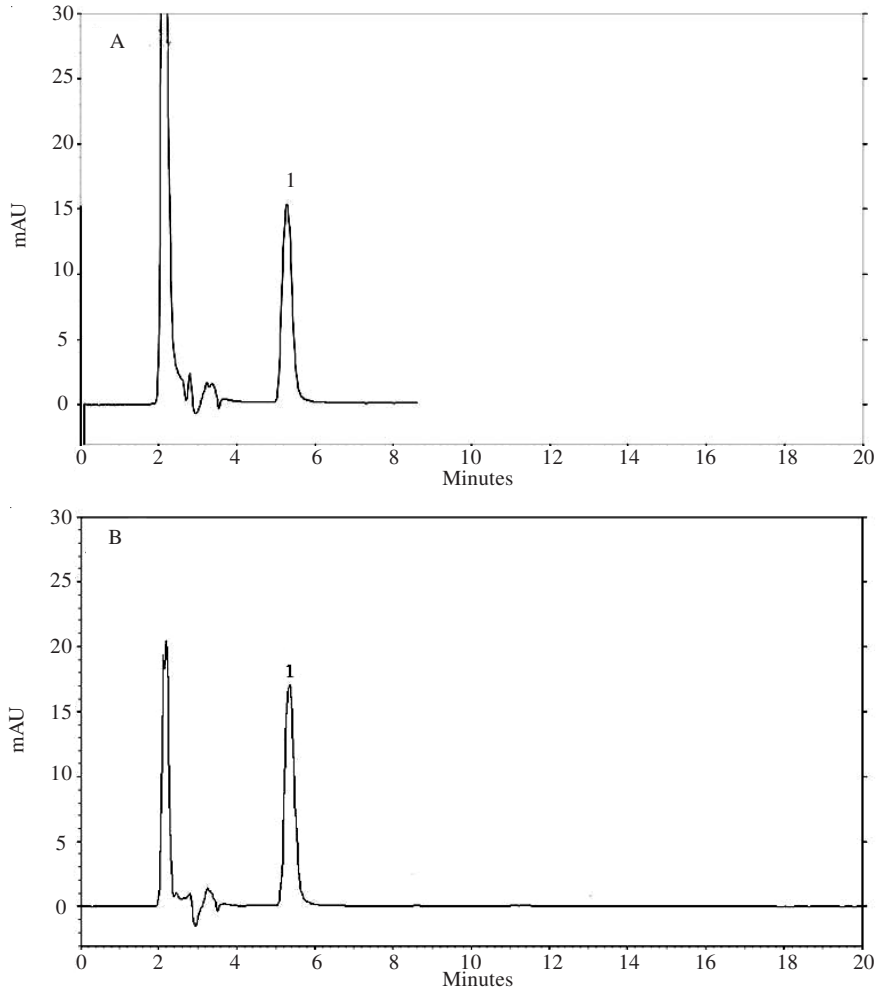


Fig. 3. Resultant chromatograms following the analysis of a standard solution of (A) CPT-11 (5 μg/mL) and (B) Camptosar® injection showing CPT-11(1)

Conclusion

In summary, a simple HPLC method is developed and validated for simultaneous determination of CPT-11, SN-38 and CPT in commercial products of CPT-11. The method is fast, efficient and has simple sample preparation procedure. The total analytical time for all analytes is less than 15 min which is an advantage for *in vitro* analysis. Rapid analyses reduce the risk of degradation and conversion of analytes during sample preparation and analysis.

ACKNOWLEDGEMENTS

The authors would like to acknowledge financial assistance from the Pharmaceutical Sciences Research Center, as well as the Novel Drug Delivery Systems Laboratories, Tehran University of Medical Sciences, Tehran, Iran, and Sobhan Chemotherapeutics Company for providing facilities used throughout this research.

REFERENCES

1. E. Gravel, P. Bourget, L. Mercier and A. Paci, *J. Pharm. Biomed. Anal*., **39**, 581 (2005).
2. E. Gupta, T.M. Lestingi, R. Mick, J. Ramirez, E.E. Vokes and M.J. Ratain, *Cancer Res.*, **54**, 3723 (1994).
3. L. Zufia, A. Aldaz and J. Giraldez, *J. Chromatogr. B Biomed*., **764**, 141 (2001).
4. A. Sparreboom, P. Bruijn, M. Jonge, W. J. Loos, G. Stoter, J. Verweij and K. Nooter, *J. Chromatogr. B: Biomed*., **712**, 225 (1998).
5. K. Sano, M. Yoshikawa, S. Hayasaka, K. Satake, Y. Ikegami, H. Yoshida, T Ishikawa, S. Sawada and S. Tanabe, *J. Chromatogr. B Biomed.*, **795**, 25 (2003).
6. Z. Hu, X. Yang, X. Chen, E. Chan, W. Duan and S.-F. Zhou, *J. Chromatogr. B: Biomed.*, **850**, 575 (2007).
7. T. Oguma, *J. Chromatogr. B Biomed*., **764**, 49 (2001).
8. T. Owens, H. Dodds, K. Fricke, S. Hanna and K. Crews, *J. Chromatogr. B: Biomed.*, **788**, 65 (2003).
9. X. Yang, Z. Hu, Y.C. Sui, B.C. Goh, W. Duan, E. Chan and S. Zhou, *J. Chromatogr. B: Biomed*., **821**, 221 (2005).
10. A. Kurita and N. Kaneda, *J. Chromatogr. B: Biomed*., **724**, 335 (1999).
11. L.P. Rivory, M. Findlay, S. Clarke and J. Bishop, *J. Chromatogr. B: Biomed*., **714**, 355 (1998).
12. S. Ragot, P. Marquet, F. Lachatre, A. Rousseau, E. Lacassie, J.M. Gaulier, J.L. Dupuy and G. Lachatre, *J. Chromatogr. B: Biomed*., **736**, 175 (1999).
13. H.M. Dodds , J. Robert and L.P. Rivory, *J. Pharm. Biomed. Anal*., **17**, 785 (1998).
14. ICH Draft Guidelines on Validation of Analytical procedures: Definitions and Terminology, Federal Register, Vol. 60, IFPMA, Switzerland (1995).
15. PDR, Thomson PDR publication, Montvale, USA, edn. 60, p. 2602 (2006).

(*Received*: 3 September 2009; *Accepted*: 23 January 2010) AJC-8360