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Irinotecan and its active metabolite, SN-38: review of bioanalytical methods and recent update from clinical pharmacology perspectives

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ABSTRACT: The introduction of irinotecan has revolutionized the applicability of camptothecins as predominant topoisomerase I inhibitor for anti-cancer therapy. The potent anti-tumor activity of irinotecan is due to rapid formation of an *in vivo* active metabolite, SN-38. Therefore, irinotecan is considered as a pro-drug to generate SN-38. Over the past decade, side-by-side with the clinical advancement of the use of irinotecan in the oncology field, a plethora of bioanalytical methods have been published to quantify irinotecan, SN-38 and other metabolites. Because of the availability of HPLC, LC-MS and LC-MS/MS methods, the pharmacokinetic profiling of irinotecan and its metabolites has been accomplished in multiple species, including cancer patients. The developed assays continue to find use in the optimization of newly designed delivery systems with regard to pharmacokinetics to promote safe and effective use of either irinotecan or SN-38. This review intends to: firstly, provide an exhaustive compilation of the published assays for irinotecan, SN-38 and other metabolite(s) of irinotecan, as applicable; secondly, to enumerate the validation parameters and applicable conclusions; and thirdly, provide some recent perspectives in the clinical pharmacology arena pertaining to efflux transporters, pediatric profiling, role of kidney function in defining toxicity, drug–drug interaction potential of irinotecan, etc. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: irinotecan; SN-38; anti-cancer; pharmacodynamics; pharmacokinetics; HPLC; LC-MS; LC-MS/MS

Introduction

Irinotecan {CPT-11, 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin, Fig. 1} is a semisynthetic analog of naturally occurring alkaloid, camptothecin (CPT, Fig. 1). Irinotecan has been marketed for the treatment of colorectal cancer for both first- and second line-therapy and other solid tumors (Potmesil, 1994). Irinotecan has shown clinical activity against colorectal, lung, gastric, cervical and ovarian cancers, malignant lymphoma and other malignancies (Ohno *et al.*, 1990; Fukuoka *et al.*, 1992; Shimada *et al.*, 1993). It acts by inhibiting DNA topoisomerase I (Topo I) (Redinbo *et al.*, 1998; Stewart *et al.*, 1998). In humans, irinotecan is metabolized by an endogenous carboxylesterase (present in intestinal mucosa, plasma and liver)-mediated hydrolyzation into a highly active metabolite (few log orders more cytotoxic than the parent molecule), SN-38 (7-ethyl-10-hydroxycamptothecin, Fig. 1), which is further converted to inactive glucuronide (SN-38G, Fig. 2) by UGT1A (uridine diphosphate glucuronosyltransferase) (de Jonge *et al.*, 1998; Iyer *et al.*, 1998). The remarkable potency of SN-38 has been instrumental for the activity of irinotecan despite the fact that SN-38 serum levels were observed to be about 100 times lower than the corresponding irinotecan levels (Catimel *et al.*, 1995). Additionally, SN-38 may also play a major role in the observed major dose-limiting toxicities including myelosuppression and unpredictable severe diarrhea generally observed with these classes of agents (Gupta *et al.*, 1994; Sugiyama *et al.*, 1998). The relationship between SN-38 and SN-38G has been shown to be a significant predictor

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Abbreviations used: ACN, acetonitrile; APC, 7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecin; $AUC_{(0-t)}$, area under the plasma concentration vs time curve from time zero to last time point measurable; $AUC_{(0-\infty)}$, area under the plasma concentration vs time curve from time zero to time infinity; AUMC, area under first moment curve; C_{max}, peak plasma concentration; CES2, carboxylesterase 2; CPT, camptothecin; CL, clearance; CPT-11, irinotecan or 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyl-oxycamptothecin; CRC, colorectal cancer; CV, coefficient of variation; CYP3A4, cytochrome P450 3A4; DCM, dichloromethane; DMEM, Dulbecco's modified Eagle's medium; F/T, freeze–thaw; HIV, human immunodeficiency virus; K_{el}, elimination rate constant; K_m, concentration of substrate that gives half-maximal activity; LLE, liquid–liquid extraction; MDR1, multidrug resistance protein 1; NPC, 7-ethyl-10-(4-amino-1-piperidino) carbonyloxy-camptothecin; P-gp, P-glycoprotein; PBS, phosphate buffer saline; PK, pharmacokinetics; RBC, red blood cells; RPMI, Roswell Park Memorial Institute; SN-38, 7-ethyl-10-hydroxycamptothecin; SN-38G, SN-38 glucuronide; SPE, solid-phase extraction; T_{max}, time to reach maximum plasma concentration; $t_{½,\beta}$, elimination half-life; TEA, triethylamine; TBAHS, tetrabutylammonium hydrogen sulfate; TBAP, tetrabutylammonium phosphate; TBME, *tert*-butyl methyl ether; Topo I, topoisomerase I; UGT, uridine diphosphate glucuronosyl-transferase 1 family polypeptide A cluster; V_d, volume of distribution; V_{max}, maximum velocity or rate at which the enzyme catalyzed a reaction; *V*ss, volume of distribution.

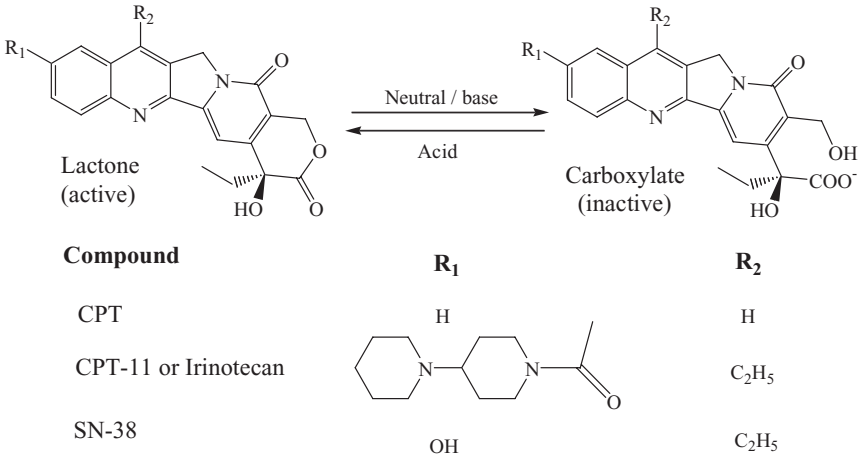

Figure 1. Structural representation of irinotecan (CPT-11), SN-38 and camptothecin (CPT).

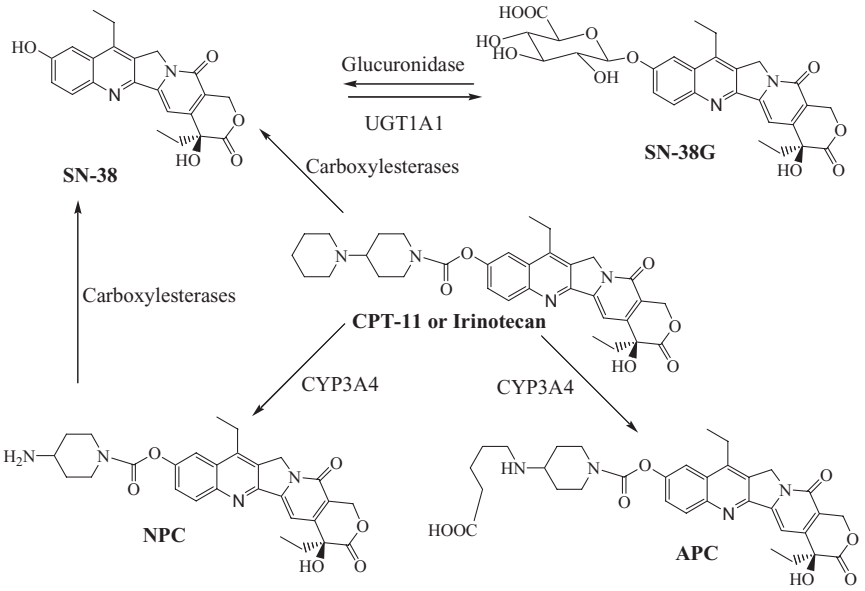

Figure 2. Major metabolic pathway of irinotecan.

of toxicity in the clinic (Gupta *et al.*, 1994). Other metabolic pathways for irinotecan include oxidation by CYP3A4 into APC (7-ethyl-10-[4-*N*-(5-aminopentanoicacid)-1-piperidino] carbonyloxycamptothecin) and NPC [7-ethyl-10-(4-amino-1-piperidino) carbonyloxycamptothecin] (Fig. 2), which are weak topoisomerase I inhibitors (Rivory *et al.*, 1996; Dodds *et al.*, 1998; Haaz *et al.*, 1998). Other CYP3A-mediated biotransformation pathways leading to the formation of hydroxylated (Lokiec *et al.*, 1996), de-ethylated (Santos *et al.*, 2000) and dehydrogenated (Sai *et al.*, 2001) metabolites have been detected in the human liver microsomes; however their biological actions or metabolic fate in humans are yet to be clarified. The lactone form of irinotecan and SN-38 has a closed α-hydroxy-δ-lactone ring, which can be hydrolyzed to form the open-ring hydroxyl acid (carboxylate form). The rate of hydrolysis of irinotecan to SN-38 is dependent on pH (Fassberg and Stella, 1992; Burke and Mi, 1993), ionic strength (Fassberg and Stella, 1992) and protein concentration (Burke and Mi, 1993; Burke *et al.*, 1995). Human serum albumin preferentially binds the carboxylate form of irinotecan over the lactone form with a 150-fold higher affinity. The lactone form has been found to be essential for the stabilization of the DNA-topoisomerase I complex and the tumor inhibitory activity of the lactone form is significantly greater than the carboxylate form (Slichenmyer *et al.*, 1993; Redinbo *et al.*, 1998; Stewart *et al.*, 1998). Previously, it was shown that only the lactone form of camptothecin analogs are able to pass cellular membranes, including those of blood cells (Loos *et al.*, 1999). The disposition characteristics of irinotecan are influenced by several drug-transporting proteins and enzyme systems, which are highly susceptible to environmental influences and genetic factors (Ma and McLeod, 2003). It should be noted that both irinotecan and SN-38 have differential binding affinity for several serum proteins (Burke and Mi, 1994; Combes *et al.*, 2000). Irinotecan, SN-38, SN-38G and APC accounted for 93% of the recovered dose in a radiolabeled mass

balance study in cancer patients (Slatter *et al*., 2000). Monitoring of total (lactone + carboxylate forms) irinotecan and total SN-38 has essentially the same clinical significance as the monitoring of lactone forms of the two agents, because the pharmacokinetics of total irinotecan and total SN-38 are significantly correlated with those of lactone irinotecan and lactone SN-38, respectively (Rivory and Robert, 1994; Rivory *et al*., 1994).

Scope

The present review has been compiled with the following specific objectives:

(1) to comprehensively perform an exhaustive literature review for assays reported for irinotecan and its metabolites inclusive of SN-38;
(2) to tabulate various validation parameters to judge the applicability and merit of the reported assays;
(3) to provide a recent update on the latest nuances reported on the clinical pharmacology aspects of irinotecan and metabolites;
(4) to provide a brief frame-work of the observed bioanaytical challenges in the assays developed for irinotecan and its metabolites.

As exemplified in Table 1, scores of analytical methods with appropriate extraction and chromatographic conditions have been used for the quantification of irinotecan and its key metabolites. Also, Table 1 covers the validation of parameters and enumerates the utility of such assays.

Clinical Pharmacology Related

Role of Efflux Transporter on the Irinotecan's Bioavailability

The role of P-glycoprotein (P-gp) in efflux-based transport has been documented for both irinotecan and SN-38 (Iyer *et al*., 2002; Itoh *et al*., 2005; Bansal *et al*., 2008b). Recently, in a preclinical model, Bansal *et al*. (2009) have studied the influence of verapamil for possible alteration of the bioavailability of both orally and intravenously administered irinotecan (Bansal *et al*., 2009). The choice of verapamil as a co-administered agent in the rat study was supported by convincing literature data on the usefulness of verapamil as an efficient P-gp blocker after *in vivo* dosing (Candussio *et al*., 2002; Choi and Li, 2005; Perez-Tomas, 2006). The oral pre-treatment with verapamil affected the bioavailability of orally administered irinotecan to a much greater extent than the intravenous (i.v.) administration of the drug (i.e. 7-fold decrease in exposure for oral vs 1.6-fold decrease in exposure of irinotecan for i.v.). Additionally, a greater impact was observed with the elimination half-life value of irinotecan for oral (prolonged by 86% from control) as compared with i.v. (prolonged by 19% from control) (Bansal *et al*., 2009). The use of verapamil as a probe substrate suggested the involvement of P-gp mechanism both at the absorption stage and in the biliary excretory pathway for irinotecan (Bansal *et al*., 2009). A separate study conducted on the biliary excretion of irinotecan in bile duct cannulated rats confirmed the significant effect of P-gp inhibition by verapamil regardless of oral or i.v. route of dosing in the rat model (Bansal *et al*., 2009). This particular study conducted in rats has greater relevance to human subjects since there is a striking similarity in the P-gp expression level (Stephens *et al*., 2001) and overlapping of substrate specificity between MDR1 identified for the two species (Yamazaki *et al*., 2001). The authors concluded that co-administration of P-gp inhibitor may be a good strategy to not only permit oral drug option for irinotecan but also reduce the diarrheal effects observed after i.v. administration of irinotecan (Bansal *et al*., 2009).

Role of Pharmacokinetics and Pharmacogenetics to Define Activity/Toxicity of Irinotecan

In an interesting study involving 49 metastatic colorectal cancer (CRC) patients, Rouits *et al*. (2008) attempted to establish relationship of pharmacokinetics (including metabolite data), non-genetic and genetic factors towards the purported toxicity and/or efficacy parameters of irinotecan (Rouits *et al*., 2008).

Since the pharmacology of irinotecan is complex in nature involving a multitude of metabolic biotransformations as well as the traditional lactone to carboxylate interconversion documented for the camptothecin class, the inter-dependence of factors such as genetic polymorphisms along with non-genetic factors are key to explaining the observed toxicity episodes and activity profile with the therapy of irinotecan in cancer patients. In this study, genetic markers included the identification of UGT1A1*28 genotype and carboxylesterase 2 (CES2) genotype in the CRC patients. The use of standard cortisol and 6β-hydroxycortisol urinary excretion pattern permitted the evaluation of the activity of CYP3A4 enzyme (Yamamoto *et al*., 2005). There was no correlation of various pharmacokinetic parameters of the parent and/or metabolites (SN-38, glucuronide of SN-38, etc.) with the genetic factors. However, the formation of APC metabolite was strongly correlated with 6β-hydroxycortisol–cortisol ratios, strongly suggesting the role of CYP3A4 in the formation of metabolite. There was a strong correlation between UGT1A1 polymorphism and the observed bilirubin plasma levels. Additionally, UGT1A1 polymorphism also predicted the occurrence of neutropenia in CRC patients. However, UGT1A1 polymorphism appeared to show no relationship for either the treatment response and/or progression-free survival of cancer patients (Rouits *et al*., 2008). The CES2 polymorphism appeared not to be correlated with a multitude of parameters (biologic, pharmacokinetics, response, etc.). However, there were few isolated relationships observed between CES2 polymorphism with diarrhea onset. The CYP3A4 status predictor, 6β-hydroxycortisol–cortisol ratio, was significantly correlated with the diarrhea occurrence (especially the 0–6 h urinary ratio) (Rouits *et al*., 2008). In summary, UGT1A1 polymorphism may be an important tool to predict hematologic toxicity (i.e. neutropenia) and the 6β-hydorxycortisol–cortisol ratio may help in gleaning the gastrointestinal toxicity (i.e. diarrhea) related to therapeutic use of irinotecan (Rouits *et al*., 2008). Interestingly, in a recently reported work, Onoue *et al*. (2008) have shown that UGT1A1 polymorphism may also be useful to predict late-onset diarrhea. While the authors have emphasized the larger role of UGT1A1 polymorphism in neutropenia prediction, the minor role of prediction of diarrhea may not be discounted (Onoue *et al*., 2008).

Role of Renal Function in Assessing Neutropenia Induced by Irinotecan

The mass balance profile of irinotecan suggests that a major portion of the administered dose in the form of derived products such as SN-38G and APC is excreted in the urine (i.e. >50% of the given dose; Sparreboom *et al*., 1998a; Slatter *et al*., 2000; de Jong *et al*., 2006). Therefore, de Jong *et al*. (2008) assessed the possible

Table 1.

Analyte(s)	Authors	Sample processing details	Chromatogrpahic conditions	Validation parameters	Applicable conclusions
Irinotecan and SN-38 (as lactones)	Bansal *et al.*, 2008a, b	Matrix: 100 μL of rat plasma and rat bile. Extraction: precipitation with cold ACN with 0.1% glacial acetic acid for plasma and DCM–TBME (3 : 7, v/v) for bile. Internal standard: topotecan (10 μL of 10 μg/mL for plasma and 10 μL of 100 μg/mL for bile).	System: HPLC with UV detector. Column: YMC C_{18} ODS-A RP (200 × 4.6 mm, 4 μm) maintained thermostatically at 35°C. Mobile phase: gradient elution comprising solvent A (ACN) and solvent B (Milli Q water with pH 3.0 adjusted with 20% *ortho*-phosphoric acid) delivered at a flow rate of 1 mL/min. Injection volume: 50 μL. Detection: irinotecan at 254 and 365 nm for plasma and bile samples, respectively. and SN-38 at 380 nm for both matrices. Retention times: 11.4, 13.4 and 15.5 min for IS, irinotecan and SN-38, respectively. Total run time: 35 min.	Regression type: linear fit. Calibration range: 25–10,000 ng/mL in plasma and 0.5–100 μg/mL in bile [correlation coefficient (r) ranged between 0.9989 and 0.9993 in plasma and bile for irinotecan and SN-38]. Limit of detection: 15 and 10 ng/mL for irinotecan and SN-38, respectively. Limit of quantitation: 40 and 20 ng/mL for irinotecan and SN-38, respectively. Absolute recovery: 93.1 ± 2.5 to 96.3 ± 1.3% for irinotecan and 92.1 ± 3.4 to 96.6 ± 2.9 for SN-38 in rat plasma; 65.1 ± 3.2 to 70.2 ± 1.5% for irinotecan and 57.4 ± 3.6 to 61.9 ± 2.6% SN-38 in rat bile. Accuracy and precision: within- and between-day precision and accuracy were found to be within acceptable limits.	The validated method was applied to determine the concentration time profiles of irinotecan and SN-38 in plasma following oral and i.v. administration of irinotecan at 80 and 20 mg/kg, respectively, to female Wistar rats. Important PK parameters viz. [$AUC_{(0-\infty)}$, T_{max}, $C0$, C_{max} V_d, Cl, K_{el} and $t_{½,\beta}$] were calculated for irinotecan and SN-38. Cumulative bile excretion of irinotecan and SN-38 up to 5 h post-dose was also studied following the above-mentioned doses administration to female Wistar rats.
Irinotecan, SN-38 and APC	D'Espositio *et al.*, 2008	Matrix: human liver microsomal preparation and human plasma. Extraction: SPE. Internal standard: CPT (50 μL, final 62.5 ng/mL).	System: LC-MS/MS with fluorescence detector. Column: Alltima C_{18} (150 × 2.1 mm, 5 μm) coupled to an Alltima C_{18} (7.5 × 2.1 mm, 5 μm) guard column. Mobile phase: gradient mobile phase comprising solvent A (water) and solvent B (ACN with 0.25% formic acid) delivered at 0.3 mL/min flow rate. Injection volume: 20 μL. Mass spectrometry detection: irinotecan: m/z 587 → 124; SN-38: m/z 393 → 349; APC: m/z 619 → 227; and IS: m/z 349 → 305. Retention times: 4.57, 4.92, 4.53, 5.09 for irinotecan, SN-38 and APC and IS, respectively. Total run time: 7 min.	Regression type: linear fit. Calibration range: 1.56–100, 3.13–150, 0.78–100 ng/mL for irinotecan, SN-38 and APC, respectively in microsomal fractions. 1.56–25, 3.13–150, 0.78–25 ng/mL for irinotecan, SN-38 and APC, respectively in plasma samples (r > 0.99). Absolute recovery: 65.77, 80.44, 67.43 and 73.58% for irinotecan, SN-38, APC and IS, respectively in microsomal fractions and 94.07, 90.47, 85.63 and 75.86% for irinotecan, SN-38, APC and IS, respectively from plasma. Selectivity: selectivity of the method was examined by analyzing 5 each of blank plasma and microsomal fractions. Endogenous interference at the retention times of the analytes was minimal. Accuracy and precision: within- and between-day precision and accuracy values found to be within the acceptable limits. Stability: irinotecan, SN-38 and APC were found to be stable in both matrices at 4°C for at least 41 days, when stored in MeOH, up to 20 h at room temperature and a period of 8 weeks at −80°C. Analytes were stable up to 5 F/T cycles in plasma.	The validated method was used to study the irinotecan biotransformation in human liver microsomal fractions in both healthy individuals and patients with liver diseases. The method has potential application in PK studies.

Table 1. *Continued*

Analyte(s)	Authors	Sample processing details	Chromatogrpahic conditions	Validation parameters	Applicable conclusions
Irinotecan and SN-38 (as lactones)	Hu *et al.*, 2007	Matrix: 100 μL of DMEM medium or H4-II-E (rat hepatoma) cancer cell line. Extraction: acidification and precipitation with methanolic IS solution. Internal standard: CPT (100 μL, final 0.1 μg).	System: HPLC with fluorescence detector. Column: Hyperclon ODS (200 × 4.6 mm) coupled to a Phenomenex C_{18} guard column. Mobile phase: ACN–50 mm disodium hydrogen phosphate buffer containing 10 mM sodium 1-heptane sulfonate (pH 3 with 85% *ortho*-phosphoric acid, w/v), 27:73, v/v with 1 mL/min flow rate. Injection volume: 20 μL. Detection: excitation and emission at 380 and 540 nm, respectively. Retention times: 10.04, 6.5 and 7.80 min for irinotecan, SN-38 and IS, respectively. Total run time: 20 min.	Regression type: linear fit. Calibration range: 5–42,000 and 2–42,000 ng/mL for irinotecan in culture media and cell lysate, respectively; 1–1500 and 0.5–1500 ng/mL for SN-38 in culture media and cell lysate, respectively ($r \geq 0.998$). Limit of detection: 1 and 0.25 ng/mL for irinotecan and SN-38, respectively in culture media and 0.5 and 0.2 ng/mL for irinotecan and SN-38, respectively in cell lysate. Absolute recovery: 96.9–108.3 and 94.3–107.2% for irinotecan in culture media and cell lysate, respectively; 87.7–106.8 and 90.1–105.6% for SN-38 in culture media and cell lysate, respectively and 90.4–103.2 and 93.2–98.7% for IS in culture media and cell lysate, respectively. Specificity: specificity was determined in presence of possible co-administered drugs viz. thalidomide, cyclophosphamide, 5-flurouracil, nifedipine, probencid, MK-571 and verapamil. Accuracy and precision: within- and between-day precision and accuracy varied from 0.1 to 10.3%.	Authors have concluded that skewed peak shape (towards right) of irinotecan was due to low pH of mobile phase and unknown factors but this did not affect the quantitation. The validated method was applied to investigate cellular metabolism and accumulation of irinotecan and SN-38 in rat hepatoma cell lines. Key enzyme kinetic parameters K_m and V_{max} for the metabolites, i.e. SN-38 and SN-38G, were calculated and it was concluded that the formation of these metabolites were following Michaelis–Menten kinetics.
SN-38 (as lactone)	Xuan *et al.*, 2006	Matrix: liposomal based SN-38 formulation (LE-SN38). Extraction: 100-fold dilution with mobile phase, no sample pretreatment or extraction is required. Internal standard: no IS was used.	System: HPLC with UV detector. Column: Zorbax SB-C_{18} ODS (250 × 4.6 mm, 5 μm) maintained thermostatically at 40°C. Mobile phase: NaH_2PO_4 (pH 3.1, 25 mM)–ACN, 50:50, v/v with 1 mL/min flow rate. Injection volume: 20 μL. Detection: UV at 265 nm. Retention time: 6 min. Total run time: 12 min.	Regression type: linear fit. Calibration range: 1–25 μg/mL ($r \geq 0.999$). Limit of detection and quantitation: 0.05 and 0.25 μg/mL. Absolute recovery: 99.9–100.7%. Specificity: lipid components and other excipients of the formulation and degradant (resulted from forced degradation studies) did not interfere with the retention of SN-38. Accuracy and precision: found to be within acceptable limits.	Authors assessed the robustness of the method (in terms of retention time and system suitability parameters) by changing the mobile phase composition, column temperature, flow rate, buffer strength and buffer pH of the mobile phase. Authors concluded that the validated method will have applicability in formulation development and preclinical studies.
Irinotecan and topotecan (as lactones)	Gravel *et al.*, 2005	Matrix: 5% dextrose infusion bags of irinotecan and topotecan. Extraction: direct injection. Internal standard: no IS was used.	System: HPLC with fluorescence detector. Column: Macherey–Nagel Nucleodur® gravity C_{18} (150 × 4.6 mm, 5 μm) maintained thermostatically at 50°C. Mobile phase: gradient elution with KH_2PO_4 (pH 3.5, 50 mM)–ACN at a flow rate of 1 mL/min. Injection volume: 100 μL. Detection: excitation and emission at 355 and 515 nm, respectively. Retention times: 2.0 and 7.0 min for topotecan and irinotecan, respectively. Total run time: 13 min.	Regression type: nonlinear fit. Calibration range: 0.1–1 mg/mL ($r^2 \geq 0.995$). Limit of detection and quantitation: 0.05 and 0.15 mg/mL, respectively for both analytes. Specificity: no interference from 5% dextrose. Accuracy and precision: found to be within acceptable limits.	Method can be used to quantitate irinotecan in infusion bags used in hospital setting for cancer patients.

Table 1. *Continued*

Analyte(s)	Authors	Sample processing details	Chromatogrpahic conditions	Validation parameters	Applicable conclusions
Irinotecan and SN-38 (as lactone and carboxylate)	Yang *et al.*, 2005	Matrix: 100 μL rat plasma. Extraction: precipitation with 200 μL of ice-cold MeOH– ACN, 1 : 1, v/v. Internal standard: CPT (100 μL of 0.05 μg CPT).	System: HPLC with fluorescence detector. Column: Hypersil C_{18} ODS (200 × 4.6 mm, 5 μm) coupled to a Phenomenex guard column. Mobile phase: isocratic mobile phase comprising 0.1 M KH_2PO_4 containing 0.01 M TBAHS (pH 6.4)–ACN, 75 : 25, v/v at a flow rate of 0.8 mL/min. Injection volume: 20 μL. Detection: excitation and emission at 380 and 540 nm, respectively. Retention times: 3.97, 5.33, 7.05, 8.12, 11.60 and 13.52 min for irinotecan carboxylate, irinotecan lactone, SN-38 carboxylate, irinotecan carboxylate, SN-38 lactone and irinotecan lactone, respectively. Total run time: 16 min.	Regression type: linear fit. Calibration range: 0.01– 10 μm ($r \geq 0.999$). Limit of quantitation: 0.01, 0.008, 0.005 and 0.05 μm for irinotecan lactone, irinotecan carboxylate, SN-38 lactone and SN-38 carboxylate, respectively. Absolute recovery: >94.4% for both lactone and carboxylate forms of irinotecan and SN-38, whereas the recovery for IS was 98.6%. Specificity: matrix-specific interference and presence of thalidomide did not affect the chromatography of the analytes. Accuracy: within-day values for irinotecan lactone, irinotecan carboxylate, SN-38 lactone and SN-38 carboxylate were 95.5–102.9, 96.4–108.8, 92.4–109.3 and 94.5–111.7%, respectively. Between-day accuracy values were 94.3–103.2, 97.3–106.2, 95.3–107.7 and 95.9–111.8%, respectively. Precision: within-day values for irinotecan lactone, irinotecan carboxylate, SN-38 lactone and SN-38 carboxylate were 1.2–4.2, 1.2–5.5, 2.0–6.4 and 1.7–5.9%, respectively. Between-day precision values were 1.3–3.6, 1.3–4.7, 1.8–5.2 and 1.3–3.9% for irinotecan lactone, irinotecan carboxylate, SN-38 lactone and SN-38 carboxylate, respectively. Dilution integrity: dilution of irinotecan in lactone or carboxylate form at 1 : 10 gave acceptable precision (CVs < 4%) and accuracy (97.3–104.4%).	All the peaks were skewed towards right side. This method was used to study the effect of co-administered thalidomide (i.p) on the PK of irinotecan and SN-38 in rats following i.v. administration of irinotecan. Co-administration of thalidomide increased (30%) the AUC values of irinotecan lactone and carboxylate but decreased (19–32%) for SN-38 lactone and carboxylate. However there was no change in the maximum plasma concentration of either irinotecan or SN-38 lactone and carboxylate forms in the presence of thalidomide. The $t_{½,\beta}$ for SN-38 lactone and carboxylate reduced more than 30%, on the other hand there was no change in the $t_{½,\beta}$ value for irinotecan lactone but for irinotecan it was increased by 49%, compared with the control group, when co-administered with thalidomide.
Irinotecan and SN-38 (as lactone and carboxylate)	Bardin *et al.*, 2005	Matrix: 100 μL mouse plasma or tissue homogenate (5% w/v in water) in a 96-well plate format. Extraction: precipitation with 200 μL ACN. Internal standard: CPT (200 μL of 10 ng/mL).	System: LC-MS/MS. Column and mobile phase: Zorbax SB-Phenyl (50 × 2 mm, 5 μm) with 20 mM ammonium acetate (pH 3.5)–ACN, 65 : 35, v/v or X-Terra C_{18} (50 × 2 mm, 5 μm) with 20 mM ammonium acetate (pH 3.5)–ACN, 67 : 33, v/v with a flow rate of 0.2 mL/min. Column was protected by 2 μm in-line filter. MRM monitoring: irinotecan: *m/z* 587.6 → 167.2; SN-38: *m/z* 393.4 → 349.3; and IS: *m/z* 349.3 → 305.3. Retention times: 1.1, 1.2 and 1.5 min for irinotecan, SN-38 and CPT, respectively. Total run time: 2.5 min.	Regression type: linear fit with weighing factor ($1/x^2$). Calibration range: 0.5–500 ng/mL for both irinotecan and SN-38 ($r \geq 0.995$). Specificity: matrix-specific interference was not observed at the retention time of the analytes and IS. Accuracy and precision: found to be within acceptable limits.	The validated method was successfully used to support PK and tissue distribution studies in mice.

Table 1. *Continued*

Analyte(s)	Authors	Sample processing details	Chromatogrpahic conditions	Validation parameters	Applicable conclusions
SN-38	Khan *et al.*, 2005	Matrix: 100 μL of mouse plasma or 200 μL of tissue homogenate. Extraction: for plasma samples—precipitation (using ACN with 10 μL 0.5% acetic acid containing 25 ng/mL of IS) followed by extraction. Tissue samples were extracted with 0.5% acetic acid containing 50 ng/mL IS followed by extraction. Internal standard: 50 μL 250 ng/mL of CPT.	System: LC-MS/MS. Column: Zorbax SB-C_{18} (50 × 2.1 mm, 5 μm) coupled to a C_{18} guard column. Mobile phase: 20 mM ammonium acetate (pH 3.5)–ACN, 65 : 35, v/v with a flow rate of 0.2 mL/min for plasma samples. For tissue samples, solvent A and solvent B were delivered at 60 : 40 ratio at a flow rate of 0.1 mL/min (solvent A, 20 mm ammonium acetate (pH 3.5)–MeOH, 80 : 20, v/v; solvent B, ACN–IPA, 75 : 25, v/v). Injection volume: 5 and 20 μL for plasma and tissue samples, respectively. Mass spectrometry detection: SN-38: m/z 393.3 → 349.1; and IS: m/z 349.1 → 305.2. Retention times: 1.3 and 1.5 for SN-38 and IS, respectively in plasma samples with a total run time of 3 min; whereas for tissue samples the total run time was 5 min with SN-38 and IS retention times of 2.7 and 3.2 min, respectively.	Regression type: linear fit with weighing factor ($1/x^2$). Calibration range: 0.5–1000 ng/mL ($r > 0.99$) for plasma and 1–400 ng/mL ($r > 0.99$) for tissue samples except for lung homogenate, where LLOQ was 2 ng/mL. Absolute recovery: 92.7 ± 1.4, 88.0 ± 11.0 and 98.8 ± 14.1% at 0.1, 10 and 200 ng/mL, respectively, whereas for IS it was 84.5 ± 1.9%. Precision and accuracy: found to be within acceptable limits. Specificity: no endogenous interference at the retention times of SN-38 and IS in both matrices. Stability: stable for 4 h at room temperature, long term for 31 days at −70°C and after three F/T cycles in plasma and tissue homogenate. Dilution integrity: linearity can be extended up to 80 and 120 μg/mL for plasma and tissue samples, respectively.	The validated method was applied to analyze plasma and tissue samples following administration of LE-SN38 (liposome entrapped SN-38) in mice
Irinotecan, SN-38, SN-38G, APC and NPC (as lactone form)	Poujol *et al.*, 2003	Matrix: 500 μL of human plasma or 100 μL human saliva. Extraction: precipitation with ACN–MeOH, 1 : 1 (1 mL for plasma or 200 μL for saliva). Internal standard: 100 μL of CPT (100 μg/L in acetone).	System: HPLC with fluorescence detector. Column: X-Terra C_{18} (250 × 4.6 mm, 5 μm) coupled with an X-Terra guard column (20 × 3.9 mm, 5 μm) maintained at ambient temperature (23°C). Mobile phase: gradient elution with solvent A (ACN–sterile water, 75 : 25, v/v) and solvent B (phosphate buffer comprising 1-heptane-sulfonic acid, pH 4.0 adjusted with *ortho*-phosphoric acid) at a flow rate of 1 mL/min. Injection volume: 100 μL at lower concentrations and 10 μL at higher concentrations. Detection: excitation was set at 370 nm and emission was set at 470 nm till 24 min (for irinotecan, SN-38G, APC and NPC) and at 534 nm for next 4 min (for SN-38). Retention times: 6.5, 13.5, 16.5, 20, 22.5 and 27 min for SN-38G, APC, NPC, irinotecan, IS and SN-38, respectively. Total run time: 30 min.	Regression type: linear fit. Calibration range: 0.5–1000 μg/mL in both matrices ($r^2 \geq 0.99$). Absolute recovery: 95.5 ± 5.4, 97.2 ± 5.9, 93.4 ± 5.0, 95.5 ± 5.8 and 99.2 ± 4.69% for irinotecan, SN-38, SN-38G, APC and NPC, respectively in human plasma, whereas in saliva the recoveries were 97.5 ± 4.2, 96.8 ± 9.5, 94.6 ± 8.2, 103.9 ± 6.3 and 100.8 ± 7.4% for irinotecan, SN-38, SN-38G, APC and NPC, respectively. Specificity: no endogenous interference at the retention time of analytes evaluated after analyzing 10 different lots of blank plasma and saliva and in presence of around 20 possible co-administered drugs. Stability: stable under battery of tests viz. short-term room temperature for 24 h, in a refrigerator at 4°C for 24 h, three F/T cycles and long-term for 4 and 6 months at −20°C (at the end of 6 months stability only for SN-38G, 37% degradation was observed at LLOQ). Authors have also established the stability for the anlaytes in acidic solution in autosampler at 23, 10, and 4°C for 48, 72, and 72 h, respectively and found that irinotecan, SN-38 and IS were stable at all conditions, whereas APC, NPC and SN-38G have shown degradation in certain conditions.	This method was used to derive the PK parameters [$AUC_{(0-t)}$, $AUC_{(0-\infty)}$, T_{max}, C_{max} and $t_{½,\beta}$] for irinotecan, SN-38, SN-38G, APC and NPC from plasma and saliva following i.v. infusion of irinotecan to patients suffering with metastatic colorectal cancer. SN-38G was not quantifiable in saliva (LLOQ: 0.75 μg/L).

Table 1. *Continued*

Analyte(s)	Authors	Sample processing details	Chromatogrpahic conditions	Validation parameters	Applicable conclusions
Irinotecan and SN-38	de Jong *et al.*, 2003	Matrix: 150 μL of blood plasma diluted RBCs (100 μL of RBCs diluted with 150 μL of human plasma). Extraction: precipitation with aqueous perchloric acid–MeOH, 1 : 1, v/v. Internal standard: no IS was used.	System: HPLC with fluorescence detector. Column: Hypersil C_{18} ODS (100 × 4.6 mm, 5 μm) maintained at 50°C. Mobile phase: isocratic mobile phase comprising MeOH–0.1 M ammonium acetate containing 0.01 M TBAHS, 35 : 60, v/v - final pH of 5.2 with HCl at a flow rate of 1.0 mL/min. Injection volume: 200 μL. Detection: excitation and emission at 355 and 515 nm, respectively. Retention times: 8.3 and 15.7 min for irinotecan and SN-38, respectively. Total run time: 20 min.	Regression type: linear fit with weighing factor ($1/x^2$). Calibration range: 5–200 ng/mL ($r \geq 0.996$). Absolute recovery: >63.3 ± 3.4 and 77.1 ± 6.9% for irinotecan and SN-38, respectively. Specificity: no endogenous interference at the retention time of analytes (evaluated after analyzing 6 different lots of blood). Accuracy: within- and between-day values of 103.4 and 105.0% for irinotecan and SN-38, respectively, in whole blood. Precision: within- and between-day values for irinotecan and SN-38 were found to be in the range of 85.2–105.7% in whole blood. In the RBCs within- and between-day values for irinotecan and SN-38 were 3.6 and 11.4 and 4.2 and 8.3%, respectively.	This method was used to study the distribution of irinotecan and SN-38 in whole blood and RBCs following 90 min i.v. infusion. The ratio of irinotecan and SN-38 in whole blood and RBCs was determined. $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ were calculated for irinotecan and SN-38 in whole blood and RBCs. Authors concluded that irinotecan distributes to RBCs, whereas SN-38 mainly localizes in the plasma compartment. The ratio of the AUC in the RBC compartment to the AUC in plasma is about 6 times higher for irinotecan compared with SN-38.
SN-38	Guo *et al.*, 2003	Matrix: 200 μL of dog plasma. Extraction: precipitation using ACN with 0.5% acetic acid. Internal standard: CPT (400 μL, 25 ng CPT/1 mL ACN).	System: HPLC with fluorescence detector. Column: Zorbax SB-C_{18} ODS (150 × 4.6 mm, 5 μm) coupled with a C_{18} guard cartridge maintained at 35°C. Mobile phase: gradient elution using mobile phase A (20 mM ammonium acetate) and B (ACN) with a flow rate of 1 mL/min. Injection volume: 45 μL. Detection: excitation and emission at 368 and 515 nm, respectively. Retention times: 6.8 and 7.9 min for SN-38 and IS, respectively. Total run time: 12 min.	Regression type: linear fit with weighing factor ($1/x$). Calibration range: 1–750 ng/mL ($r \geq 0.999$). Absolute recovery: 78 ± 4.5 and 86 ± 7.2% for SN-38 and IS, respectively. Specificity and matrix effect: no endogenous interference at the retention time of analytes (evaluated after analyzing 6 different lots of plasma). No matrix effect was observed. Accuracy: within- and between-day values were 96.6–104 and 96.5–103%, respectively. Precision: within- and between-day values were <7.09 and 3.22%, respectively. Dilution integrity: calibration curve can be extended up to 60,000 ng/mL with out affecting final concentration. Stability: stable under battery of tests viz. room temperature (4 h), three F/T cycles and long-term for 57 days at −70°C.	The validated method was successfully used to quantify SN-38 following i.v. administration of LE-SN38 (liposomal preparation) to beagle dogs in preclinical and toxicokinetic studies.
Irinotecan and SN-38 (total as carboxylate forms)	Schoemaker *et al.*, 2003	Matrix: 100 μL of human plasma. Extraction: precipitation with 200 μL of ACN–MeOH (1 : 1, v/v) Internal standard: no IS was used.	System: HPLC with fluorescence detector. Column: Zorbax SB-C_{18} (150 × 4.6 mm, 3.5 μm) coupled with a Chrompack guard column (10 × 3 mm) maintained at 40–45°C. Mobile phase: isocratic mobile phase comprising 0.1 mol/L ammonium acetate– TEA–ACN, 800 : 1 : 200, v/v delivered at a flow rate of 1.5 mL/min. Injection volume: 25 μL.	Regression type: linear fit. Calibration range: 5–1500 and 0.5–100 ng/mL for irinotecan and SN-38, respectively ($r \geq 0.99$). Absolute recovery: 109 ± 3.6 and 106 ± 5.1% for irinotecan and SN-38, respectively. Specificity and matrix effect: no endogenous interference at the retention time of analytes from control plasma. Morphine, caffeine and acetaminophen did not interfere with the assay.	Authors used the basic pH (9.0) conditions to measure the total as carboxylate form. This method was used to quantify irinotecan and SN-38 following i.v. administration of irinotecan infusion.

Table 1. *Continued*

Analyte(s)	Authors	Sample processing details	Chromatogrpahic conditions	Validation parameters	Applicable conclusions
			Detection: excitation at 385 nm and emission at 535 nm. Retention times: 5.2 and 9.4 min for irinotecan and SN-38, respectively. Total run time: 12 min.	Accuracy and precision: The intra-assay and inter-assay accuracy for all tested concentrations were within ± 15% and the precisions were less than ± 15%. Stability (for both irinotecan and SN-38): stable under battery of tests viz. room temperature (24 h), three F/T cycles in whole blood and plasma, autosampler (4°C) for 5 days and long-term for 1.8 years at −20°C.	
Irinotecan, SN-38, SN-38G and APC (both lactone and carboxylate forms)	Owens *et al.*, 2003	Matrix: 460 µL of human plasma. Extraction: precipitation using cold MeOH. Internal standard: no IS was used.	System: HPLC with fluorescence detector. Column: Symmetry C_8 (150 × 3.9 mm, 5 µm) coupled with a Symmetry C_8 guard column (20 × 3.9 mm, 5 µm). Mobile phase: gradient elution using mobile phase A (86% of 0.75 M ammonium acetate , 5 mM TBAP, pH 6 and 14% ACN) and B (50% of 0.75 M ammonium acetate, 5 mM TBAP, pH 6 and 50% ACN) with a flow rate of 1.25 mL/min. Injection volume: 100 µL. Detection: excitation at 380 nm and emission at 460 and 530 nm as per the each compound optimal wavelength. Retention times: 4.7, 8.9, 11.7, 14.2, 16.2, 18.2, 22.1 and 23.6 min for SN-38 G carboxylate, APC carboxylate, irinotecan carboxylate, SN-38 G lactone, APC lactone, irinotecan lactone, SN-38 carboxylate and SN-38 lactone, respectively. Total run time: 40 min (though all analytes eluted within 25 min extended run time of 40 min was used to get a stable base line before next injection).	Regression type: linear fit with weighing factor (1/*x*). Calibration range: 5–300 ng/mL [correlation coefficient (*r*) ranged between 0.953 and 0.995]. Limit of quantitation: 0.5 ng/mL for SN-38 lactone, 1 ng/mL for SN-38 carboxylate; 2 ng/mL for APC-lactone, APC-carboxylate, SN-38G lactone, SN-38G carboxylate and 5 ng/mL for irinotecan lactone and irinotecan carboxylate. Absolute recovery: ranged between 82 and 117% for all the analytes. Specificity and matrix effect: no endogenous interference at the retention time of analytes (evaluated after analyzing 6 different lots of plasma). No matrix effect was observed. Accuracy: within- and between-day accuracy values for all the analytes ranged between 79.2 and 117.6%. Precision: at low concentrations the intra- and inter-day RSD values were ≤11.5 and ≤18.6%, respectively, whereas at high concentration the intra- and inter-day RSD values were ≤15 and ≤11.7%, respectively.	Authors discussed numerous variables effect on method development and simultaneous estimation of lactone and carboxylate forms of the analytes in a single run. This method was used to quantify irinotecan and its metabolites viz. SN-38, SN-38G and APC following i.v. administration of irinotecan infusion.
SN-38 (total as lactone form)	Khan *et al.*, 2003	Matrix: 200 µL of human hepatic microsomes reaction mixture or human plasma. Extraction: precipitation using cold 1 mL ACN with 10 µL 0.5% acetic acid. Internal standard: 50 µL 250 ng/mL of CPT.	System: LC-MS/MS Column: Synergy Hydro-RP C_{18} (50 × 2 mm, 4 µm). Mobile phase: gradient elution using ACN– 0.1% acetic acid. Injection volume: 5 µL. Mass spectrometry detection: SN-38: *m/z* 393.1 → 349.2; and IS: *m/z* 349.1 → 305.1. Retention times: 1.55 and 1.78 for SN-38 and IS, respectively. Total run time: 3 min.	Regression type: linear fit with weighing factor (1/*x*). Calibration range: 0.05–400 ng/mL ($r > 0.997$). Absolute recovery: 92.7 ± 1.4, 88.0 ± 11.0 and 98.8 ± 14.1% at 0.1, 10 and 200 ng/mL, respectively, whereas for IS it was 84.5 ± 1.9%. Selectivity: no endogenous interference at the retention time of analytes (evaluated after analyzing 6 different lots of male and female human subject's plasma) at the retention time of analyte and IS. Precision and accuracy: found to be within acceptable limits.	This method has potential application in a clinical pharmacokinetic study.

Table 1. *Continued*

Analyte(s)	Authors	Sample processing details	Chromatogrpahic conditions	Validation parameters	Applicable conclusions
				Stability: stable for 7.75 h at room temperature and after three F/T cycles. Found to be stable in reconstitution solvent at room temperature for 80.25 h. Dilution integrity: dilution of SN-38 in lactone form at 1 : 200 gave acceptable precision (CVs < 12.7%) and accuracy percentage of −10.8 to 1.25.	
Irinotecan, SN-38, SN-38G and APC	Sai *et al.*, 2002	Matrix: 200 μL of human hepatic microsomes reaction mixture or human plasma. Extraction: precipitation using 5% perchloric acid–MeOH (1 : 1). Internal standard: 10 μL 100 ng/mL of CPT.	System: HPLC coupled parallel to both fluorescence and mass spectrometry (LC-MS) detectors. Column: Capcell Pak CN UG120 (150 × 4.6 mm, 5 μm) coupled with a NewGuard C_8 guard column maintained at 50°C. Mobile phase: isocratic mobile phase comprising ACN– MeOH–0.05 M ammonium acetate (pH 4.5), 14 : 14 : 72 delivered at a flow rate of 0.5 mL/min. Fluorescence detection: excitation at 368 nm and emission at 432 nm for and irinotecan, SN-38G, APC, NPC and for SN-38 the excitation and emission were set at 368 and 535 nm, respectively. Mass spectrometry detection: selected ions monitored as protonated molecules $(M + H)^+$ were *m/z* 569 for SN-38G, *m/z* 559 for M1 (deethylated), *m/z* 519 for NPC, *m/z* 603 for M2 (hydroxylated), *m/z* 619 for APC, *m/z* 585 for M3 (dehydrogenated), *m/z* 587 for irinotecan, *m/z* 349 for CPT and *m/z* 393 for SN-38. Retention times: 4.7, 8.9, 11.7, 14.2, 16.2, 18.2, 22.1 and 23.6 min for SN-38 G carboxylate, APC carboxylate, irinotecan carboxylate, SN-38 G lactone, APC lactone, irinotecan lactone, SN-38 carboxylate and SN-38 lactone, respectively. Total run time: 20 min.	Regression type: linear fit with weighing factor (1/*x*). Calibration range: 2.5–800 ng/mL for SN-38 ($r \geq 0.996$) and APC ($r \geq 0.999$); 5–4000 ng/mL for irinotecan ($r \geq 0.999$) and 1–80 ng/mL for SN-38G ($r \geq 0.997$) on fluorescence detector. In case of LC-MS the ranges of linearity were 10–800 ng/mL for SN-38G ($r \geq 0.997$) and APC ($r \geq 0.999$); 10–4000 ng/mL irinotecan ($r \geq 0.999$) and 2–80 ng/mL for SN-38 ($r \geq 0.985$). Limit of quantitation: 2.5 ng/mL for SN-38G and APC; 5 ng/mL for irinotecan and 1 ng/mL for SN-38 with fluorescence detection. Absolute recovery: >80% for irinotecan, SN-38 and APC; 101% for SN-38G. Selectivity: no endogenous interference at the retention time of analytes (evaluated after analyzing 6 different lots of plasma) and 18 other drugs, which are likely to be co-administered with irinotecan. Precision: at low concentrations the intra- and inter-day RSD values were below 6 and 7–22% on fluorescence and MS detectors, respectively; whereas at high concentration the intra- and inter-day RSD values were less than 4 and 10% on fluorescence and MS detectors, respectively. Stability (for irinotecan, SN-38, SN-38G and APC): stable after five F/T cycles and on long-term storage for 4 weeks at −80°C.	Authors demonstrated that maintaining pH of 4.5 or storage at 4°C prevents the degradation of analytes at least 24 h, hence the mobile phase pH was adjusted to 4.5 and autosampler was maintained at 4°C. There was no significant difference between containers, i.e. glass, polypropyrene or siliconized tubes, in the relative changes of the standards dissolved in PBS for the 6 weeks storage at −80°C. Irinotecan calibration curve was used to quantify the metabolites formed from *in vitro* systems after establishing that the precision and accuracy values were within the acceptable limits. Method was applied to study the metabolic pattern of irinotecan in human microsomal system.
Irinotecan and SN-38 (both lactone and carboxylate forms)	Boyd *et al.*, 2001	Matrix: tissue culture media and HT29 and HCT116 cell lines. Extraction: SPE. Internal standard: no IS was used.	System: HPLC with fluorescence detector. Column: Symmetry Shield RP18 (150 × 3 mm, 5 μm) coupled with a Waters guard column (20 × 3.8 mm, 5 μm) maintained at 35°C. Mobile phase: gradient elution using mobile phase A (10 mM ammonium acetate) and B (MeOH) with a flow rate of 0.35 mL/min. Injection volume: 20–50 μL.	Regression type: linear fit. Calibration range: 1–400 ng/mL ($r^2 \geq 0.998$). Limit of detection: 1, 4, 3 and 1 pg/mL for irinotecan lactone, irinotecan carboxylate, SN-38 lactone and SN-38 carboxylate, respectively. Limit of quantitation: 0.2, 2, 0.5 and 2 ng/mL for irinotecan lactone, irinotecan carboxylate, SN-38 lactone and SN-38 carboxylate, respectively.	Usage of Symmetry columns reduced the peak broadening and tailing. Authors discussed the stability of irinotecan and SN-38 lactone and carboxylic acids in various buffers and different temperature conditions. This new method was used to evaluate the accumulation of irinotecan and SN-38 in HT29 and HCT116 human cancer cells. In HT29 cells formation of SN-38G was observed.

Table 1. *Continued*

Analyte(s)	Authors	Sample processing details	Chromatogrpahic conditions	Validation parameters	Applicable conclusions
			Detection: excitation at 380 nm and emission at 423 nm. Retention times: 9.5, 10.5, 11, 15 and 16 min for SN-38G, irinotecan carboxylate, SN-38 carboxylate, irinotecan lactone and SN-38 lactone, respectively. Total run time: 21 min.	Absolute recovery: 99.6 ± 10.1 and 98.0 ± 5.1% for irinotecan and SN-38 lactones, respectively and 81.0 ± 16.9 and 77.3 ± 9.0% for irinotecan and SN-38 carboxylates, respectively from tissue culture media. Specificity: endogenous interference from cell lines or interfering peaks from media did not interfere with chromatography of irinotecan and SN-38. Accuracy and precision: found to be within the acceptable limits.	
Irinotecan and SN-38 (lactone form)	Escoriaza *et al.*, 2000	Matrix: 100 µL of human plasma. Extraction: precipitation with 100 µL of IS solution and 200 µL of ACN–1 mm of *ortho*-phosphoric acid (90 : 10). Internal standard: 100 µL of 1 µg/mLof CPT [in ACN–1 mm of *ortho*-phosphoric acid (90 : 10)].	System: HPLC with fluorescence detector. Column: Nucleosil C_{18} (250 × 4 mm, 5 µm) coupled with a Nucleosil C_{18} pre-column (4 × 4 mm, 5 µm) maintained at 30°C. Mobile phase: isocratic mobile phase comprising 0.1 M KH_2PO_4 (pH 4.2 with 1 m HCl)–ACN, 67 : 33 at a flow rate of 1 mL/min. Injection volume: 100 µL. Detection: excitation was fixed at 228 nm, whereas the emission was set at 450, 543 and 433 nm for irinotecan, SN-38 and CPT, respectively. Retention times: 4.57, 6.83, and 8.44 min for irinotecan, SN-38 G and CPT, respectively. Total run time: 10 min.	Regression type: linear fit. Calibration range: 1–10,000 ng/mL for irinotecan and 0.5–400 ng/mL for SN-38. Absolute recovery: 93.5 ± 4.11, 93.1 ± 4.35 and 93.8 ± 3.11% for irinotecan, SN-38 and CPT, respectively. Selectivity: no interfering peaks were observed from plasma pools and patient sample at the retention time of analytes. Accuracy and precision: found to be within the acceptable limits. Stability: processed samples were found to be stable in autosampler for 24 h.	Addition of 0.1 m KH_2PO_4 (pH 4.2) buffer to the processed plasma samples improved the peak shape. This method was used to determine the concentrations of irinotecan, SN-38 and SN-38G, determined following i.v. infusion of irinotecan. Various PK parameters viz. $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, T_{max}, C_{max}, $t_{½,\beta}$, Cl, V_{ss} and AUMC were calculated for irinotecan, whereas for SN-38 and SN-38G only $AUC_{(0-t)}$, C_{max} and T_{max} were calculated.
Irinotecan and SN-38 (both lactone and carboxylate forms)	Ragot *et al.*, 1999	Matrix: 200 µL human serum for irinotecan and 1 mL of human serum for SN-38. Extraction: protein precipitation followed by acidification (20 µL of sodium citrate, 2.5 M, pH 2) for irinotecan and LLE for SN-38 with 200 µL ACN. Internal standard: CPT (50 µL of 1 mg/L).	System: LC-MS using eletrospray ionization. Column and mobile phase: Symmetry C_{18} (150 × 1 mm, 3.5 µm) with gradient elution of ACN in 5 mM ammonium formate buffer (pH 3) at a flow rate of 50 µL/min. Column was maintained thermostatically at 35°C. Injection volume: 2 µL. Ions used for quantification: *m/z* 587.3, 605.3, 393, 411.2 and 349.2 for irinotecan lactone, irinotecan carboxylate, SN-38 lactone, SN-38 carboxylate and IS, respectively. Retention times: 3.5, 3.9, 5.1, 5.4 and 5.5 min for irinotecan carboxylate, irinotecan lactone, SN-38 lactone, IS and SN-38 carboxylate, respectively. Total run time: 10 min.	Regression type: linear fit with weighing factor (1/x). Calibration range: 10–10000 and 0.5–100 ng/mL for both irinotecan and SN-38, respectively ($r \geq 0.999$). Limit of detection: 2.5 and 0.25 ng/mL for irinotecan and SN-38, respectively. Limit of quantitation: 10 and 0.5 ng/mL for irinotecan and SN-38, respectively. Specificity: matrix-specific interference was not observed at the retention time of the analytes and IS. Carboxylate forms of the analytes did not interfere with the lactones retention time. Recovery: 68.0 ± 7.3, 70.5 ± 10.0 and 68.9 ± 5.3% at 50, 1000 and 5000 ng/mL, respectively, for irinotecan; 48.6 ± 3.2, 43.8 ± 1.7 and 49.6 ± 3.7% at 5, 25 and 75 ng/mL , respectively, for SN-38; 86.6 ± 6.7 and 73.5 ± 10.0% for IS by protein precipitation and LLE, respectively. Accuracy and precision: found to be within acceptable limits for both the analytes (measured as lactone form).	The suitability of the validated method was demonstrated following i.v. infusion of irinotecan to a cancer patient to determine the concentrations of irinotecan and SN-38 as lactone forms in serum.

Table 1. *Continued*

Analyte(s)	Authors	Sample processing details	Chromatogrpahic conditions	Validation parameters	Applicable conclusions
Irinotecan, SN-38 and SN-38G (as lactone form)	Kurita and Kaneda, 1999	Matrix: 50 μL of rat plasma. Extraction: automated SPE following acidification with 0.146 M of H_3PO_4. Internal standard: 250 μL of 1 μg/mL CPT.	System: HPLC with fluorescence detector. Column: Symmetry C_{18} (150 × 4 mm, 5 μm) maintained at 50°C. Mobile phase: isocratic mobile phase comprising 0.05 M KH_2PO_4–ACN, 70:30, v/v containing 4 mM sodium 1-decanesulfate (pH 3.5 with H_3PO_4) at a flow rate of 1.5 mL/min. Detection: excitation and was set at 373 and 428 nm, respectively for SN-38G (from 0–2.7 min); excitation and emission at 380 and 540 nm, respectively, from 2.7 to 3.8 min for SN-38 and from 3.8 to 8.5 min the detector was set at 373 and 428 nm as excitation and emission, respectively, for irinotecan and CPT. Retention times: 1.5, 3.2, 4.2 and 6.7 min for SN-38G, SN-38, CPT and irinotecan, respectively. Total run time: 9 min.	Regression type: linear fit. Calibration range: 5–25,000 ng/mL for irinotecan; 5–2500 ng/mL for SN-38 and 2.5–500 ng/mL for SN-38G ($r \geq 0.9999$ or better). Absolute recovery: 96.0–117.3, 97.0–106.8 and 96.2–119.6% for irinotecan, SN-38 and SN-38G, respectively. Selectivity: no interfering peaks were observed at the retention time of analytes. Though little interference was observed at the retention time of SN-38G, it did not interfere with the determination of SN-38G. Accuracy: within-day accuracy values were 4.0, 6.6 and 8.1%, respectively for irinotecan, SN-38 and SN-38G, respectively, whereas between-day values were 3.3, 3.7 and 10.5% for irinotecan, SN-38 and SN-38G, respectively. Precision: within-day for irinotecan at LLOQ was 14.2 and 1.9% at higher concentrations, whereas for SN-38 and SN-38G the values were 1.6 and 3.4%, respectively. Between-day values were 7.63, 2.4 and 5.0% for irinotecan, SN-38 and SN-38G, respectively. Stability: stable during three F/T cycles. Following acidification of plasma samples the stability of irinotecan, SN-38 and SN-38G stability was assessed following incubation at 37°C for 24 h and found that all the analytes were stable, whereas the non-addition of acid following incubation has resulted in degradation of 19 and 4% for irinotecan and SN-38G, the degradation of SN-38 was 40-fold.	This method applicability has been shown in quantification of irinotecan, SN-38 and SN-38G following i.v. administration of irinotecan at different doses. This method directly quantifies SN-38G.
SN-38	de Bruijn *et al.*, 1997	Matrix: 1 mL of human plasma. Extraction: precipitation followed by LLE. Internal standard: 100 μL of CPT (100 ng/mL in MeOH: 0.01 M aqueous HCl, 2:3, v/v).	System: HPLC with fluorescence detector. Column: Hypersil ODS (100 × 4.6 mm, 5 μm) maintained thermostatically at 60°C. Mobile phase: ACN–0.1 M ammonium acetate containing 10 mm tetra-butyl ammonium sulfate (23:77, v/v), pH 5.3 with HCl at a flow rate of 1 mL/min. Injection volume: 75 μL. Detection: excitation and emission wavelength set at 380 and 556 nm, respectively. Retention times: 4.9 and 5.5 min for SN-38 and IS, respectively. Total run time: 15 min.	Regression type: linear fit with weighing factor ($1/x^2$). Calibration range, 5–2000 pg/mL ($r \geq 0.995$). Lower limit of quantitation: 0.25 and 0.5 ng/mL and 1.0 and 2.0 ng/mL for irinotecan and SN-38 lactone and total forms, respectively. Selectivity: there was no interference at the retention times of SN-38 and IS from endogenous components of plasma, APC, NPC and also from the 12 other drugs, which may be clinically administered, pre- or post-chemotherapy of SN-38. Absolute recovery: 88.3 ± 14.3 and 94.8 ± 3.9% for SN-38 and IS, respectively. Stability: stable after repeated F/T cycles and the processed samples were stable at room temperature for overnight.	The described method will be used in future studies to assess the extent of enterohepatic recirculation of SN-38 in cancer patients following i.v. irinotecan treatment.

Table 1. *Continued*

Analyte(s)	Authors	Sample processing details	Chromatogrpahic conditions	Validation parameters	Applicable conclusions
Irinotecan and SN-38 (lactone and carboxylate forms)	Chollet *et al.*, 1998	Matrix: 100 µL of rat and dog plasma. Extraction: precipitation with IS solution and ACN–1 mm of *ortho*-phosphoric acid (90 : 10, pH 3). Internal standard: no IS used.	System: HPLC with fluorescence detector. Column: Symmetry C_{18} (150 × 3.9 mm, 5 µm). Other columns like LC- ABZ column (150 × 4.6 mm, 5 µm) and Nucleosil CN, C_8 and C_{18} (125 × 4.0 mm, 5 µm) were also used. Mobile phase: gradient elution using mobile phase A (0.075 M ammonium acetate + 7.5 mM tetra-butyl ammonium bromide, pH 6.4 with glacial acetic acid) and B (ACN) with a flow rate of 1.8 mL/min. Injection volume: 10 µL. Detection: the detection program for dog plasma samples analysis: at 0 min, ex 362 nm and em 425 nm; 7.2 min, ex 375 nm and em 560 nm; and 17 min, ex 362 nm and em 425 nm. Detection program for rat plasma samples analysis: at 0 min, ex 362 nm and em 425 nm; 11.8 min, ex 375 nm and em 560; and 17 min, ex 362 nm and em 425. Retention times: 4.1, 6.1, 8.9 and 10.4 min for irinotecan lactone, irinotecan carboxylate, SN-38 lactone and SN-38 carboxylate, respectively in dog plasma and 6.4, 7.7, 10.6, 12.5 and 14.0 min for irinotecan lactone, irinotecan carboxylate, SN-38 lactone and SN-38 carboxylate, respectively in rat plasma. Total run time: 20 min.	Regression type: linear fit with weighing factor ($1/x$ or $1/x^2$). Calibration range: 0–1.604, 0–1.27 nmoL/mL for irinotecan (lactone and carboxylate) and SN-38 (lactone and carboxylate), respectively ($r \geq 0.999$). Absolute recovery: 88.6 ± 4.7 and 85.6 ± 3.1% for irinotecan (lactone and carboxylate) and SN-38 (lactone and carboxylate), respectively in dog plasma. The recovery in rat plasma was 77.1 ± 7.3 and 89.8 ± 5.9% for irinotecan (lactone and carboxylate) and SN-38 (lactone and carboxylate), respectively. Selectivity: no endogenous interference observed. Accuracy and precision: found to be within the acceptable limits except for SN-38 carboxylate in dog plasma. Authors could not found the valid reasons for this deviation. Stability: processed samples were found to be stable in autosampler for 24 h.	The stability of irinotecan and SN-38 lactone and carboxylate forms was assessed in 0.5 m phosphate buffer solutions at different pH conditions under darkness at 25°C for 20 h and their stability established. Authors also established that, during sample collection, lactone and carboxylate forms of the analytes did not have equilibrium displacement or interchangeable hydrolysis of the two forms. The validated method was successfully applied to PK and toxicokinetic studies in rats and dogs following i.v. infusion of irinotecan.
SN-38 (latone form)	Rivory *et al.*, 1998	Matrix: 100 µL human plasma. Extraction: SPE and acidification. Internal standard: CPT (100 µL of 5 nM).	System: HPLC with fluorescence detector. Column: Nova-Pak Radial-Pak C_{18} (100 × 5 mm, 5 µm) coupled to a guard column (Guard-Pak, Nova-Pak). Mobile phase: ACN–0.075 M ammonium acetate buffer (pH 5.3), 23 : 77, v/v delivered at a flow rate of 1.5 mL/min. Injection volume: 25 µL. Detection: excitation was set at 380 for both SN-38 and IS, whereas the emission was set at 532 and nm for SN-38 and IS, respectively, to maximize the response. Retention times: 4.0 and 5.1 min for SN-38 and IS, respectively. Total run time: 8 min.	Regression type: linear fit with weighing factor ($1/x^2$). Calibration range: 10–500 pm or 3.9–195 pg/mL ($r^2 \geq 0.996$). Selectivity: during SPE clean-up the cartridges were eluted with MeOH and this has avoided the endogenous interference at the achieved LLOQ (10 pM). Absolute recovery: 48.3 ± 15.8 and 91.5 ± 4.5% at 10 and 500 pM, respectively for SN-38 and 96.9 ± 6.4 for IS at 500 pM. Accuracy and precision: within-day accuracy and precision ranged between 93.8–108.9 and 1.8–16.5%, respectively, whereas the between-day accuracy and precision ranged between 91.4–106.4 and 3.3–16.2%, respectively.	This method was applied to estimate the trough concentrations of SN-38 following administration of irinotecan. Authors could estimate SN-38 levels before second cycle commencement. During the analysis irinotecan was not detected.

Table 1. *Continued*

Analyte(s)	Authors	Sample processing details	Chromatogrpahic conditions	Validation parameters	Applicable conclusions
Irinotecan, SN-38, SN-38G, and APC (lactone form)	Sparreboom *et al.*, 1998b	Matrix: 250 μL of human plasma or 250 μL plasma diluted urine (1 : 1, v/v). Feces samples were treated with perchloric acid and homogenized. Following centrifugation, the supernatant was processed like urine sample. Extraction: LLE with aqueous perchloric acid (5% w/v)–MeOH, 1 : 1, v/v. Internal standard: no IS was used.	System: HPLC with fluorescence detector. Column: Hypersil ODS (100 × 4.6 mm, 5 μm) coupled to a guard column (LiChroCART, 4 × 4 mm, 5 μm) maintained at 50°C. Mobile phase: MeOH: 0.1 m ammonium acetate containing tetrabutyl ammonium sulfate (30 : 70, v/v, pH 5.3 with HCl). Injection volume: 100–200 μL. Detection: excitation and emission wavelength set at 355 and 515 nm, respectively. Retention times: 7.08, 12.7, 17.9 and 29.8 min for SN-38G, APC, irinotecan and SN-38, respectively. Total run time: 35 min.	Regression type: linear fit with weighing factor ($1/x^2$). Calibration range: 2–200 ng/mL for plasma ($r \geq 0.998$) and 100–5000 ng/mL for urine ($r \geq 0.998$) and feces ($r \geq 0.9991$). Lower limit of quantitation: 0.25 and 0.5 and 1.0 and 2.0 ng/mL for irinotecan and SN-38 lactone and total forms, respectively. Selectivity: plasma collected from the patient prior drug administration did not endogenous interference at the retention times of the analytes. Absolute recovery: 95.0 ± 4.3, 82.6 ± 4.2, 85.3 ± 5.3 and 99.3 ± 9.2% in plasma; 98.9 ± 11.2, 9.7 ± 4.6, 89.2 ± 12.4 and 98.1 ± 6.5% in urine and 110.5 ± 8.3, 100.5 ± 5.1, 101.5 ± 9.3 and 113.6 ± 4.7% in feces for SN-38G, APC, irinotecan and SN-38, respectively. Accuracy and precision: found to be within the acceptable limits.	Following samples processing before injecting into HPLC system for analysis, authors diluted the supernatant in the mobile phase, which helped in baseline separation of a few analytes, improved extraction efficiency and decreased endogenous interference. The validated method was used in a Phase-I clinical and PK study of irinotecan in combination with cisplatin. Irinotecan was administered as an i.v. infusion. Blood, urine and feces samples were collected up to 48 h to perform PK analysis.
Irinotecan and SN-38 (both lactone and total forms)	de Bruijn *et al.*, 1997	Matrix: 250 and 1000 μL of human plasma for the quantitation of total and lactone forms, respectively (for both analytes). Extraction: precipitation with aqueous perchloric acid–ACN, 1 : 1, v/v for the estimation of total form. LLE for the estimation of lactone form. Internal standard: CPT 100 μL of 25 ng/mL in MeOH–0.01 m aqueous HCl (2 : 3, v/v).	System: HPLC with fluorescence detector. Column: Hypersil ODS (100 × 4.6 mm, 5 μm) coupled to a guard column (LiChroCART, 4 × 4 mm, 5 μm) maintained at 50°C. Mobile phase: MeOH: 0.1 M ammonium acetate containing tetrabutyl ammonium sulfate (40 : 60, v/v) for the lactone form and 35 : 65, v/v (pH 5.5 with HCl) for total form. Injection volume: 100 μL. Detection: excitation and emission wavelength set at 355 and 515 nm, respectively. Retention times: 4.9, 6.5 and 8.1 min for lactone form of irinotecan, CPT and SN-38, respectively; 8.3 and 15 min for total form of irinotecan and SN-38, respectively. Total run time: 10 and 20 min for lactone and total forms, respectively.	Regression type: linear fit with weighing factor ($1/x^2$). Calibration range: 0.5–2000 and 2–750 ng/mL for lactone and total forms (for both analytes), respectively ($r \geq 0.995$). Lower limit of quantitation: 0.25 and 0.5 ng/mL and 1.0 and 2.0 ng/mL for irinotecan and SN-38 lactone and total forms, respectively. Selectivity: plasma collected from the patient prior drug administration did not endogenous interference at the retention times of the analytes. Absolute recovery: 91.5 ± 3.9, 87.5 ± 8.7, 85.3 ± 5.3, 99.3 ± 9.2 and 88.1 ± 5.1% for irinotecan lactone, SN-38 lactone, irinotecan total, SN-38 total and IS, respectively. Accuracy and precision: found to be within the acceptable limits. Between-day and within-day precision values for both the analytes (both forms) were within 15%, whereas the average accuracy ranged between −13.1 and +12.2%. Stability: stable under battery of tests viz. autosampler (20 h), five F/T cycles and long-term for 2 months at −80°C.	The validated method was applied to phase I clinical PK study of irinotecan along with cisplatin in a cancer patient. Irinotecan was administered as an i.v. infusion.

Table 1. *Continued*

Analyte(s)	Authors	Sample processing details	Chromatogrpahic conditions	Validation parameters	Applicable conclusions
SN-38 (lactone and carboxylate forms)	Kaneda *et al*., 1997	Matrix: 100 µL of rat plasma. Extraction: precipitation with chilled MeOH and zinc sulfate. Internal standard: no IS was used.	System: HPLC with fluorescence detector. Column: Puresil C_{18} (150 × 4.6 mm, 5 µm) coupled to a guard column. Mobile phase: 0.1 M ammonium acetate (pH 5.5)–ACN, 70:30, v/v containing 20 mm tetra-*n*-pentyl-ammonium bromide at a flow rate of 1 mL/min. Detection: excitation and emission wavelength set at 380 and 540 nm, respectively. Retention times: 4.5 and 5.8 min for SN-38 lactone and carboxylate, respectively. Total run time: ~8 min.	Regression type: linear fit with weighing factor ($1/x^2$). Calibration range: 5–1000 and 5–2500 ng/mL for lactone and carboxylate of SN-38, respectively ($r^2 \geq 0.989$ and 0.999 for lactone and carboxylate forms, respectively). Selectivity: though endogenous peaks were observed around the retention time of lactone form, they did not affect the determination of lactone form. Absolute recovery: 85.2 ± 1.2 and 83.6 ± 2.6% for lactone and carboxylate, respectively. Precision: intra-day precision value (CV%) for lactone at LLOQ and higher concentrations was 14.6 and 5.1%, respectively and that for carboxylate was 10.8% at LLOQ and 4.9% at higher concentrations. Inter-day precision values for lactone and carboxylate were 13.7 and 14.7%, respectively. Accuracy: intra-day accuracy values (% RE) for lactone and carboxylate were within 7.2 and 4.3%, respectively. Inter-day values for lactone and carboxylate were 13.8 and 7.2%, respectively. Stability: lactone form was found to be stable for 6 h on ice and at room temperature, whereas carboxylate form was found to degrade by 72.6 and 57.7% on ice and at room temperature, respectively. When stored for 72 h at −30°C and −80°C, lactone form (96.2%) was more stable than carboxylate form (89.3%). As the injection solvent contained 50% MeOH, the carboxylate form easily converting to lactone form at room temperature and on ice.	During the sample preparation and chromatography, there was no inter-conversion of lactone to carboxylate and vice versa.
Irinotecan and SN-38 (lactone form)	Sumiyoshi *et al*., 1995	Matrix: 200 µL of human plasma. Extraction: LLE with MeOH. Internal standard: CPT (50 µL of 1.25 µg/mL).	System: HPLC with fluorescence detector. Column: TSK gel ODS- 80Ts (150 × 4.6 mm, 5 µm) coupled to a guard column (TSK guradgel ODS-120T, 15 = 3.2 mm, 5 µm) maintained at 30°C. Mobile phase: ACN–50 mM disodium hydrogen sulfate (28:72, v/v) with 5 mm hetpane sulfonate (pH 3.0). Injection volume: 100 µL. Detection: excitation and emission wavelength set at 380 and 556 nm, respectively. Retention times: 5.4, 7.3 and 8.8 min for lactone form of irinotecan, SN-38 and IS, respectively.	Regression type: linear fit with weighing factor ($1/x^2$). Calibration range: 30–2000 and 1–30 ng/mL for irinotecan and SN-38, respectively. Lower limit of quantitation: 0.25 and 0.5 ng/mL and 1.0 and 2.0 ng/mL for irinotecan, SN-38 lactone and total forms, respectively. Selectivity: plasma collected from the patient prior drug administration did not endogenous interference at the retention times of the analytes. Absolute recovery: 87, 90 and 90% for irinotecan, SN-38 and IS, respectively.	The validated method was applied to a PK study of irinotecan and SN-38 in patients with small-cell lung cancer following administration of irinotecan as an i.v. infusion (60 mg/m^2).

Table 1. *Continued*

Analyte(s)	Authors	Sample processing details	Chromatogrpahic conditions	Validation parameters	Applicable conclusions
			Total run time: 12 min.	Accuracy and precision: found to be within the acceptable limits. Between-day and within-day precision values for both the analytes (both forms) were within 15%, whereas the average accuracy ranged between −13.1 and +12.2%. Stability: stable under battery of tests viz. autosampler (20 h), five F/T cycles and long-term for 2 months at −80°C.	
Irinotecan and SN-38 (carboxylate and lactone forms)	Rivory and Robert, 1994	Matrix: 50 μL of human plasma. Extraction: precipitation with 100 μL of ice-cold methanol: ACN, 1 : 1, v/v mixture. Internal standard: 50 μL of CPT (0.05 μg).	System: HPLC with fluorescence detector. Column: Nova-Pak Radial-Pak C_{18} (100 × 5 mm, 4 μm) coupled to a guard column (Guard-Pak, Nova-Pak, C_{18}). Mobile phase: ACN–0.075 M ammonium acetate buffer (22 : 78, v/v) with 5 mm tetrabutyl ammonium phosphate delivered at a flow rate of 1.5 mL/min. Injection volume: 5–20 μL. Detection: excitation and emission wavelength set at 355 and 515 nm, respectively. Retention times: 4.1, 5.2 and 6.5 min for carboxylate form of irinotecan, SN-38 and IS, respectively; 8.2, 9.3 and 10.4 min for lactone form of irinotecan, SN-38 and IS, respectively. Total run time: 12 min.	Regression type: linear fit with weighing factor ($1/x^2$). Calibration range: 10 ng/mL to 12.5 μg/mL for irinotecan lactone and carboxylate; 2 ng/mL to 2.5 μg/mL for both SN-38 lactone and carboxylate ($r \geq 0.999$). Absolute recovery: 93.8 ± 2.1 to 110.9 ± 10.6 and 89.6 ± 17.3 to 105.1 ± 3.4 for irinotecan carboxylate and lactone, respectively; 94.6 ± 2.1 to 132.2 ± 29.6 and 90.4 ± 5.8 and 105.8 ± 15.1% for SN-38 carboxylate and lactone, respectively, whereas the recovery for IS was 101.1 ± 5.8%. Accuracy and precision: found to be within the acceptable limits. Stability: stable under battery of tests viz. autosampler (20 h), five F/T cycles and long-term for 2 months at −80°C.	Authors briefly discussed method optimization to remove the interference of unknown peaks and importance of IS having structural similarity to the analytes. This method was used to derive the PK parameters of irinotecan and SN-38 in patients following administration of irinotecan i.v. infusion. During the analysis of patient plasma samples, authors noticed the presence of SN-38 carboxylate (2.3 min) and lactone (2.9 min) peaks.
Irinotecan and SN-38 (as lactone form)	Barilero *et al.*, 1992	Matrix: 100 μL of human plasma. Extraction: SPE. Before subjecting for SPE treatment plasma was treated with 850 μL of 0.01 M HCl. Internal standard: CPT (50 μL of 1 μg/mL).	System: HPLC with fluorescence detector. Column: Nucleosil ODS (300 × 3.9 mm, 10 μm) coupled to a guard column (Nucleosil, 22 × 3.5 mm, 10 μm). Mobile phase: isocratic mobile phase comprising ACN–0.1 M KH_2PO_4 (34 : 66, v/v) with 3 mM sodium hetpane sulfonate (pH 4.0 with 1 M HCl). Detection: excitation and emission wavelength set at 380 and 500 nm, respectively. Retention times: 5.5, 7.2 and 9.0 min for irinotecan, SN-38 and IS, respectively. Total run time: 10 min.	Regression type: linear fit. Calibration range: 1 ng/mL to 10 μg/mL ($r^2 \geq 0.9997$ and 0.9958 for irinotecan and SN-38, respectively). Absolute recovery: 84 ± 8, 99 ± 3 and 72 ± 4% for irinotecan, SN-38 and IS, respectively. Accuracy and precision: within-day precision RSDs were 13 and 12.8% for irinotecan and SN-38, respectively; whereas the mean accuracies were 103.5 and 94.9% for irinotecan and SN-38, respectively. The mean between-day RSDs were 7.9 and 9.7% for irinotecan and SN-38, respectively; with accuracies of 106.4 and 94.3% for irinotecan and SN-38, respectively.	The validated method was applied to generate the plasma concentration–time profiles for irinotecan and SN-38 following i.v. infusion of irinotecan. Key PK parameters viz. V_d, *Cl* and AUC were determined.

ACN, acetonitrile; APC, 7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecin; $AUC_{(0-t)}$, area under the plasma concentration vs time curve from time zero to last time point measurable; $AUC_{(0-\infty)}$, area under the plasma concentration vs time curve from time zero to time infinity; C_{max}, peak plasma concentration; CPT, camptothecin; CV, coefficient of variation; DCM, dichloromethane; DMEM, Dulbecco's modified Eagle's medium; ex, excitation; em, emission; HCl, hydrochloric acid; H_3PO_4, phosphoric acid; i.p, intraperitoneal; i.v., intravenous; K_m, concentration of substrate that gives half-maximal activity; KH_2PO_4, potassium dihydrogenphosphate; LLE, liquid–liquid extraction; MeOH, methanol; NPC, 7-ethyl-10-(4-amino-1-piperidino)carbonyloxycamptothecin; PBS, phosphate buffer saline; PK, pharmacokinetics; *r*, correlation coefficient; r^2, coefficient of determination; RBC, red blood cells; RE, relative error; RSD, relative standard deviation; SN-38, 7-ethyl-10-hydroxycamptothecin; SN-38G, SN-38 glucuronide; SPE, solid-phase extraction; T_{max}, time for the attainment; $t_{½,\beta}$, elimination half-life; TBAHS, tetrabutylammonium hydrogen sulfate; TBAP, tetrabutylammonium phoshphate; TBME, *tert*-butyl methyl ether; TEA, triethylamine; UV, ultraviolet; V_d, volume of distribution; V_{max}, maximum velocity or rate at which the enzyme catalyzed a reaction.

role of renal function to aid in the prediction of irinotecan-induced drug toxicity. In the study, clinical data from about 131 patients with standard recommended doses of irinotecan provided direct evidence of the association of renal function with both occurrence and gradation of hematological toxicity (i.e. neutropenia) (de Jong *et al.*, 2008). In particular, the association of grades 3 or 4 neutropenia tended to directly correlate with the individual's drop in creatinine clearance. For example in patients with creatinine clearance of 35–66 mL/min, almost 58% of the patients manifested grade 3 or 4 neutropenia in sharp contrast to patients with normal creatinine clearance (>130 mL/min), where only 14% of patients showed grade 3 or 4 neutropenia (de Jong *et al.*, 2008). The data from this recently published study suggest that one has to pay attention to renal function, especially for irinotecan and other CPT analogs (de Jong *et al.*, 2008).

Pediatric Pharmacokinetics of Irinotecan and Metabolites

Thompson *et al.* (2008) reported a population pharmacokinetic model for irinotecan and four metabolites. The modeling exercise carried out in this work was supported by data from 82 patients (1–21 years of age). Both systemic clearance (CL) and steady-state volume of distribution (V_{ss}) were scaled using allometric approaches. In the model, age and bilirubin levels (pre-treatment) served as co-variates (Thompson *et al.*, 2008). While the developed model was shown to have striking similarity when compared with the adult population pharmacokinetic model that was previously reported for irinotecan (Klein *et al.*, 2002), it also independently confirmed that co-variates of age and bilirubin were important to predict toxicity of irinotecan (Thompson *et al.*, 2008). The pediatric model unambiguously supported the current daily dosing schedule used for irinotecan in pediatric patients (Thompson *et al.*, 2008).

Drug–drug Interaction Potential of Irinotecan in HIV Patients

Corona *et al.* (2008) have shown clinically meaningful drug–drug interaction when lopinavir–ritonavir combination was co-administered with irinotecan in HIV patients afflicted with Kaposi's sarcoma. This study involved detailed evaluation of the pharmacokinetics of irinotecan and its major metabolites when irinotecan was given by itself and in the presence of liponavir–ritonavir combination to HIV patients. The exposures of irinotecan (i.e. $AUC_{0-\infty}$) increased by almost 2-fold and that of SN-38 increased by almost 3-fold upon the co-administration of irinotecan with the anti-retroviral combination therapy of lopinavir–ritonavir (Corona *et al.*, 2008). In both instances, the half-life value for either irinotecan or SN-38 was not affected by the combination (Corona *et al.*, 2008). The formation of SN-38 glucuronide conjugate was also increased by 2-fold without any alteration in the half-life by the liponavir–ritonavir combination (Corona *et al.*, 2008). However, APC exposure was drastically reduced by almost 6-fold without any noticeable change in the half-life value due to the co-administration of the liponavir–ritonavir combination. The authors postulated that liponavir–ritonavir combination could inhibit multiple excretory pathways controlled by UGT1A1 and P-gp efflux pumps, besides the traditional CYP3A4 system. Additionally, greater than 2-fold accumulation of parent and key metabolites (SN-38 and SN-38 conjugate) may pose significant safety risks if patients are not closely monitored and/or if dose reduction of irinotecan is not instituted (Corona *et al.*, 2008).

Bioanalytical Considerations

Avoidance of Plasma Interference

Although not reported for irinotecan and/or SN-38, Liu *et al.* (2008) found an unusual interference when they used a protein precipitation method to process the incurred samples of an analog of CPT and they attributed this interference to an unidentified metabolite. Interestingly, they were able to completely eliminate the recovery of the interference peak by a careful selection of a specific organic solvent. In this example, six organic solvents namely of *t*-butylmethylether, dichloromethane, diethyl ether, ethyl acetate, *n*-hexane and isopropanol were evaluated and diethyl ether was chosen as the solvent of choice (Liu *et al.*, 2008).

Peak Shape Issues

Hu *et al.* (2007) observed that shape of peaks corresponding to SN-38 and internal standard (CPT) were slightly skewed to the right (asymmetry coefficients were mild ranging between 1.03 and 1.10 (Hu *et al.*, 2007). However, the peak corresponding to irinotecan was drastically skewed (asymmetry coefficients were between 1.15 and 1.31). The authors did not rectify the peak shape since the quantitative aspects of irinotecan, SN-38, and/or internal standard appeared to be least affected by the observed peak shapes. The authors speculated that the low pH of the mobile phase may have contributed for the observed asymmetry in peak shapes (Hu *et al.*, 2007). An earlier work of Poujol *et al.* (2003) also observed somewhat higher skewness in the peak shapes of both irinotecan (asymmetry coefficient of 1.27) and APC (asymmetry coefficient of 1.23) (Poujol *et al.*, 2003).

Contamination of Plasma with Red Blood Cells

De Jong *et al.* (2003) carried out an interesting study that demonstrated that irinotecan but not SN-38 had the propensity to be taken up preferentially by red blood cells (RBC) in human cancer patients. Therefore, one has to be cognizant that hemolyzed plasma and/or hemolyzed serum may artifactually inflate the concentration of irinotecan. However, de Jong *et al.* (2003) noted that small contamination of RBC with plasma (approximately 3–4%) should not affect the quantitative determination of either irinotecan or SN-38 (de Jong *et al.*, 2003).

Stability Concerns for Key Metabolites

The work of Poujol *et al.* (2003) sheds some interesting perspectives with regard to stability problems of the key metabolites of irinotecan. APC was found to be most unstable among the metabolites of irinotecan. When stored and/or taken up by acidic extracts, a dramatic reduction of approximately 39–50% of the absolute area was noted within 24 h at ambient temperature. Although the degradation of APC was minimized when the temperature was held at 10°C, it still accounted for 10–22% losses within a day. Interestingly, the loss of APC appeared to be correlated with the increase in NPC levels, suggesting a partial conversion of APC into NPC in acidic extracts. The glucuronic acid conjugate of SN-38 (SN-38G) in acidic extracts showed stability at the lowest spiked level at ambient temperature for 48 h. In contrast, the two higher spiked levels of SN-38G showed massive degradation (approximately 80% loss) within 24 h at ambient temperatures. However, the use of lower temperatures (4 and

10°C) appeared to render SN-38G stable for at least 72 h (Poujol *et al.*, 2003).

SN-38-related Stability Issues

In order to quantify the intracellular levels of SN-38 lactone form, Sano *et al.* (2003) had to determine the stability of SN-38 in various solutions to permit the HPLC determination of the SN-38 solution. The various solutions that were used in this experiment included: water (pH 6), PBS (pH 7.4), human plasma (pH 7.4) and RPMI 1640 medium (pH 7.4). In water SN-38 appeared to be stable for a 3 h period, but in other media there was a rapid loss of SN-38 (>50% loss within 1 h) (Sano *et al.*, 2003). During the 3 h duration, the loss of SN-38 was >95% for the RPMI media and ranged between 75 and 90% loss for PBS and human plasma systems (Sano *et al.*, 2003).

Inter-conversion of Lactone to Carboxylate Forms

The work of Sano *et al.* (2003) succinctly described the inter-conversion phenomenon occurring between lactone and carboxylate forms of new CPT derivatives, including SN-38. The data also suggested a significant accumulation of both forms of CPT in the intracellular targets, although the levels of lactone form appeared to be generally higher (Sano *et al.*, 2003). Regardless of the type of CPT analog, the lactone form was stable at pH 6.0 and below. As the pH increased from 6.0, the conversion of lactone to carboxylate form became evident and at pH 8.0 and above the predominant species present was the carboxylate form of CPT (Sano *et al.*, 2003). It should be noted that, although lactone form was stable at pH 6.0, a negligible conversion of carboxylate to lactone form still continued to occur until it reached pH 3.0 (Sano *et al.*, 2003). Earlier work of Boyd *et al.* (2001) confirmed the importance of pH in the inter-conversion between lactone and carboxylic acid forms of CPTs. While pH 5.0 showed the greatest stability, pH 9.0 showed rapid conversion of lactone to carboxylate forms within a few hours (Boyd *et al.*, 2001). It was also shown that chilling samples at 4°C could perhaps help to sustain the stability of lactone for approximately 6 h even at a physiologically relevant pH of 7.4 (Boyd *et al.*, 2001).

Discussion

Along with topotecan, irinotecan has been the only other topoisomerase I inhibitor in the market for over a decade now. Irinotecan has not only filled the important void of an active cytotoxic agent with a different mechanism of action, but also provides combination opportunities with other existing drugs and/or with other investigational drugs in various treatment modalities for cancer. The availability of a plethora of completely validated bioanalytical methods has paved the way for characterization of the pharmacokinetic profile of irinotecan in various preclinical species and human subjects. Therefore, depending on the sensitivity needs and objectives of the investigational protocol including the intended matrix there is an opportunity to select a specific methodology (HPLC vs LC-MS/MS) applicable to irinotecan alone, irinotecan in combination with SN-38 or irinotecan in combination with SN-38 and other metabolites.

As it is a typical requirement in the modern day bioanalysis field, it is important to ensure that specificity issues do not interfere in the performance of the assay, especially if one adopts a published validated method for irinotecan and/or its metabolites. Therefore, additional validation experiments may be needed to address the interference issue(s) if other co-medications are given along with irinotecan in cancer patients. Such additional experiments may have to be planned to address both chromatography interference and mass spectral interference. Also, specifically related to LC-MS/MS assays, one needs to be cognizant of whether or not other co-medications are contributing towards causing significant matrix effects and if so, appropriate measures are put in place to minimize and/or eliminate such matrix effects (Srinivas, 2009a).

Future Perspectives

The repository of bioanalytical methodology and its associated nuances published on irinotecan, SN-38 and other metabolites may find use in adapting to an appropriate assay procedure without having to spend significant amounts of time and resources on method development/validation activities. Since many delivery devices including oral formulations of SN-38 with advanced technologies are being explored, the listed assays in this review would come in handy to characterize the pharmacokinetic attributes and verify the appropriateness of such novel delivery systems and/or formulations to achieve the intended systemic exposure level. As always, the availability of LC-MS/MS assays for the bioanalysis of irinotecan may also aid in easy adaptation of the assay if newer metabolite(s) of either irinotecan and/or SN-38 is identified. It may also be possible that the sensitivity limits of the assays for irinotecan and/or SN-38 may be further improved to quantify the intracellular levels of the drug and/or active metabolites in resistant tumor models, which may have an over-expressed transporter system(s). In this regard the summation approaches of multiple MRM transition pairs in LC-MS/MS may greatly aid in the improvization of sensitivity needed for intracellular determination of irinotecan and/or its metabolites (Srinivas, 2009b; Manjunath Swamy *et al.*, 2009).

References

Bansal T, Awasthi A, Jaggi M, Khar RK and Talegaonkar S. Development and validation of reversed phase liquid chromatographic method utilizing ultraviolet detection for quantification of irinotecan (CPT-11) and its active metabolite, SN-38, in rat plasma and bile samples: application to pharmacokinetic studies. *Talanta* 2008a; **76**: 1015–1021.

Bansal T, Awasthi A, Jaggi M, Khar RK and Talegaonkar S. Pre-clinical evidence for altered absorption and biliary excretion of irinotecan (CPT-11) in combination with quercetin: possible contribution of P-glycoprotein. *Life Sciences* 2008b; **83**: 250–259.

Bansal T, Awasthi A, Jaggi M, Khar RK and Talegaonkar S. Effect of P-glycoprotein inhibitor, verapamil, on oral bioavailability and pharmacokinetics of irinotecan in rats. *European Journal of Pharmaceutical Sciences* 2009; **36**: 580–590.

Bardin S, Guo W, Johnson JL, Khan S, Ahmad A, Duggan JX, Ayoub J and Ahmad I. Liquid chromatographic–tandem mass spectrometric assay for the simultaneous quantification of Camptosar and its metabolite SN-38 in mouse plasma and tissues. *Journal of Chromatography A* 2005; **1073**: 249–255.

Barilero I, Gandia D, Armand JP, Mathieu-Boue A, Re M, Gouyette A and Chabot GG. Simultaneous determination of the camptothecin analogue CPT-11 and its active metabolite SN-38 by high-performance liquid chromatography: application to plasma pharmacokinetic studies in cancer patients. *Journal of Chromatography B* 1992; **575**: 275–280.

Boyd G, Smyth JF, Jordell JI and Cummings J. High-performance liquid chromatographic technique for the simultaneous determination of lactone and hydroxy acid forms of camptothecin and SN-38 in tissue

culture media and cancer cells. *Analytical Biochemistry* 2001; **297**: 15–24.
Burke TG and Mi Z. Preferential binding of the carboxylate form of camptothecin by human serum albumin. *Analytical Biochemistry* 1993; **212**: 285–287.
Burke TG and Mi Z. The structural basis of camptothecin interactions with human serum albumin: impact on drug stability. *Journal of Medicinal Chemistry* 1994; **37**: 40–46
Burke TG, Munshi CB, Mi Z and Jiang Y. The important role of albumin in determining the relative human blood stabilities of the camptothecin anticancer drugs. *Journal of Pharmaceutical Sciences* 1995; **84**: 518–519.
Candussio L, Decorti G, Crivellato E, Granzotto M, Rosati A, Giraldi T and Bartoli F. Toxicology and pharmacokinetic study of low doses of verapamil combined with doxorubicin. *Life Sceinces* 2002; **71**: 3109–3119.
Catimel G, Chabot GG, Guastalla JP, Dumortier A, Cote C, Engel C, Gouyette A, Mathieu-Boué A, Mahjoubi M and Clavel M. Phase I and pharmacokinetic study of irinotecan (CPT-11) administered daily for three consecutive days every three weeks in patients with advanced solid tumors. *Annals of Oncology* 1995; **6**: 133–140.
Choi JS and Li X. The effect of verapamil on the pharmacokinetics of paclitaxel in rats. *European Journal of Pharmaceutical Sciences* 2005; **24**: 95–100.
Chollet DF, Goumaz L, Renard A, Montay G, Vernillet L, Arnera V and Mazzo DJ. Simultaneous determination of the lactone and carboxylate forms of the camptothecin derivative CPT-11 and its metabolite SN-38 in plasma by high-performance liquid chromatography. *Journal of Chromatography B* 1998; **718**: 163–175.
Combes O, Barre J, Duche JC, Vernillet L, Archimbaud Y, Marietta MP, Tillement JP and Urien S. In vitro binding and partitioning of irinotecan (CPT-11) and its metabolite, SN-38, in human blood. *Investigational New Drugs* 2000; **18**: 1–5.
Corona G, Vaccher E, Sandron S, Sartor I, Tirelli U, Innocenti F and Toffoli G. Lopinavir–ritonavir dramatically affects the pharmacokinetics of irinotecan in HIV patients with Kaposi's sarcoma. *Clinical Pharmacology and Therapeutics* 2008; **83**: 601–606.
de Bruijn P, Verweij J, Loos WJ, Nooter K, Stoter G and Sparreboom A. Determination of irinotecan (CPT-11) and its active metabolite SN-38 in human plasma by reversed-phase high-performance liquid chromatography with fluorescence detection. *Journal of Chromatography B* 1997; **698**: 277–285.
D'Esposito F, Tattam BN, Ramzan I and Murray M. A liquid chromatography/electrospray ionization mass spectrometry (LC-MS/MS) assay for the determination of irinotecan (CPT-11) and its two major metabolites in human liver microsomal incubations and human plasma samples. *Journal of Chromatography B* 2008; **875**: 522–530.
de Jong FA, Mathiissen RH, de Bruijn P, Loos WJ, Verweij J and Sparreboom A. Determination of irinotecan (CPT-11) and SN-38 in human whole blood and red blood cells by liquid chromatography with fluorescence detection. *Journal of Chromatography B* 2003; **795**: 383–388.
de Jong FA, Kitzen JJ, de Bruijn P, Verweij J and Loos WJ. Hepatic transport, metabolism and biliary excretion of irinotecan in a cancer patient with an external bile drain. *Cancer Biology and Therapy* 2006; **5**: 1105–1110.
de Jong FA, van der Bol JM, Mathijssen RH, van Gelder T, Wiemer EA, Sparreboom A and Verweij J. Renal function as a predictor of irinotecan-induced neutropenia. *Clinical Pharmacology and Therapeutics* 2008; **84**: 254–262.
de Jonge MJA, Sparreboom A and Verweij J. The development of combination therapy involving camptothecins: a review of preclinical and early clinical studies. *Cancer Treatment Reviews* 1998; **24**: 205–220.
Dodds HM, Haaz MC, Riou JF, Robert J and Rivory LP. Identification of a new metabolite of CPT-11 (irinotecan): pharmacological properties and activation to SN-38. *Journal of Pharmacology and Experimental Therapeutics* 1998; **286**: 578–583.
Escoriaza J, Aldaz A, Castellanos C, Calvo E and Giraldez J. Simple and rapid determination of irinotecan and its metabolite SN-38 in plasma by high-performance liquid-chromatography: application to clinical pharmacokinetic studies. *Journal of Chromatography B* 2000; **740**: 159–168.
Fassberg J and Stella VJ. A kinetic and mechanistic study of the hydrolysis of camptothecin and some analogues. *Journal of Pharmaceutical Sciences* 1992; **81**: 676–684.
Fukuoka M, Niitani H, Suzuki A, Motomiya M, Hasegawa K, Nishiwaki Y, Kuriyama T, Ariyoshi Y, Negoro S and Masuda N. A phase II study of CPT-11, a new derivative of camptothecin, for previously untreated non-small-cell lung cancer. *Journal of Clinical Oncology* 1992; **10**: 16–20.
Gravel E, Bourget P, Mercier L and Paci A. Fluorescence detection combined with either HPLC or HPTLC for pharmaceutical quality control in a hospital chemotherapy production unit: application to camptothecin derivatives. *Journal of Pharmaceutical and Biomedical Analysis* 2005; **39**: 581–586.
Guo W, Yang XX, Chen X, Duan W and Zhou SF. Determination by liquid chromatography with fluorescence detection of total 7-ethyl-10-hydroxy-camptothecin (SN-38) in beagle dog plasma after intravenous administration of liposome-based SN-38 (LE-SN38). *Journal of Chromatography B* 2003; **791**: 85–92.
Gupta E, Lestingi TM, Mick R, Ramirez J, Vokes EE and Ratain MJ. Metabolic fate of irinotecan in humans: correlation of glucuronidation with diarrhea. *Cancer Research* 1994; **54**: 3723–3725.
Haaz MC, Rivory C, Riche C, Vernillet L and Robert J. Metabolism of irinotecan (CPT-11) by human hepatic microsomes: participation of cytochrome P-450 3A and drug interactions. *Cancer Research* 1998; **58**: 468–472.
Hu ZP, Yang XX, Chen X, Chan E, Duan W and Zhou SF. Simultaneous determination of irinotecan (CPT-11) and SN-38 in tissue culture media and cancer cells by high performance liquid chromatography: application to cellular metabolism and accumulation studies. *Journal of Chromatography B* 2007; **850**: 575–580.
Itoh T, Itagaki S, Sumi Y, Hirano T, Takemoto I and Iseki K. Update of irinotecan metabolite SN-38 by the human intestinal cell line Caco-2. *Cancer Chemotherapy and Pharmacology* 2005; **55**: 420–424.
Iyer L, King CD, Whitington PF, Green MD, Tephly TR, Coffman BL and Ratain MJ. Genetic predisposition to the metabolism of irinotecan (CPT-11). Role of uridine diphosphate glucuronosyltransferase isoform1A1 in the glucuronidation of its active metabolite (SN-28) in human liver microsomes. *Journal of Clinical Investigations* 1998; **101**: 847–854.
Iyer L, Ramírez J, Shepard DR, Bingham CM, Hossfeld DK, Ratain MJ and Mayer U. Biliary transport of irinotecan and metabolites in normal and P-glyco- protein-deficient mice. *Cancer Chemotherapy and Pharmacology* 2002; **49**: 336–341.
Kaneda N, Hosokawa Y and Yokokura T. Simultaneous determination of the lactone and carboxylate forms of 7-ethyl-10-hydroxycamptothecin (SN-38), the active metabolite of irinotecan (CPT-11), in rat plasma by high performance liquid chromatography. *Biological and Pharmaceutical Bulletin* 1997; **20**: 815–819.
Khan S, Ahmad A and Ahmad I. A sensitive and rapid liquid chromatography tandem mass spectrometry method for quantitative determination of 7-ethyl- 10-hydroxycamptothecin (SN-38) in human plasma containing liposome- based SN-38 (LE-SN38). *Biomedical Chromatography* 2003; **17**: 493–499.
Khan S, Ahmad A, Guo W, Wang YF, Abu-Qare A and Ahmad I. A simple and sensitive LC/MS/MS assay for 7-ethyl-10-hydroxycamptothecin (SN-38) in mouse plasma and tissues: application to pharmacokinetic study of liposome entrapped SN-38 (LE-SN38). *Journal of Pharmaceutical and Biomedical Analysis* 2005; **37**: 135–142.
Klein CE, Gupta E, Reid JM, Atherton PJ, Sloan JA, Pitot HC, Ratain MJ and Kastrissios H. Population pharmacokinetic model for irinotecan and two of its metabolites, SN-38 and SN-38 glucuronide. *Clinical Pharmacology and Therapeutics* 2002; **72**: 638–647.
Kurita A and Kaneda N. High-performance liquid chromatographic method for the simultaneous determination of the camptothecin derivative irinotecan hydrochloride, CPT-11, and its metabolites SN-38 and SN-38 glucuronide in rat plasma with a fully automated on-line solid-phase extraction system, PROSPEKT. *Journal of Chromatography B* 1999; **724**: 335–344.
Liu X, Wang L, Vardeman D, Cao Z and Giovanella B. Development and validation of a reverse-phase HPLC with fluorescence detector method for simultaneous determination of CZ48 and its active metabolite camptothecin in mouse plasma. *Journal of Chromatography B* 2008; **867**: 84–89.
Lokiec F, du Sorbier BM and Sanderink GJ. Irinotecan (CPT-11) metabolites in human bile and urine. *Clinical Cancer Research* 1996; **2**: 1943–1949.
Loos WJ, Verweij J, Gelderblom HJ, de Jonge MJ, Brouwer E, Dallaire BK and Sparreboom A. Role of erythrocytes and serum proteins in the kinetic profile of total 9-amino-20(*S*)-camptothecin in humans. *Anticancer Drugs* 1999; **10**: 705–710.
Ma MK and McLeod HL. Lessons learned from the irinotecan metabolic pathway. *Current Medicinal Chemistry* 2003; **10**: 41–49.

Manjunath Swamy J, Kamath N, Anil Kumar, Shekar R, Srinivas NR and Kristijansson F. Sensitivity enhancement and matrix effect evaluation during summation of multiple transition pairs—case studies of clopidogrel and ramiprilat. *Biomedical Chromatography* 2009; DOI: 10.1002/bmc.1322.

Ohno R, Okada K, Masaoka T, Kuramoto A, Arima T, Yoshida Y, Ariyoshi H, Ichimaru M, Sakai Y and Oguro M. An early phase II study of CPT-11: a new derivative of camptothecin, for the treatment of leukemia and lymphoma. *Journal of Clinical Oncology* 1990; **8**: 1907–1912.

Onoue M, Kurita A, Kado S, Matsumoto T, Kaneda N, Uchida K, Kato I and Yokokura T. Involvement of UDP-glucuronosyltransferase activity in irinotecan-induced delayed-onset diarrhea in rats. *Cancer Chemotherapy and Pharmacology* 2008; **61**: 595–605.

Owens TS, Dodds H, Fricke K, Hanna SK and Crews KR. High-performance liquid chromatographic assay with fluorescence detection for the simultaneous measurement of carboxylate and lactone forms of irinotecan and three metabolites in human plasma. *Journal of Chromatography B* 2003; **788**: 65–74.

Perez-Tomas R. Multidrug resistance: retrospect and prospects in anticancer drug treatment. *Current Medicinal* Chemistry 2006; **13**: 1859–1876.

Potmesil M. Camptothecins: from bench research to hospital wards. *Cancer Research* 1994; **54**: 1431–1439.

Poujol S, Pinguet F, Malosse F, Astre C, Ychou M, Culine S and Bressolle F. Sensitive HPLC-fluorescence method for irinotecan and four major metabolites in human plasma and saliva: application to pharmacokinetic studies. *Clinical Chemistry* 2003; **49**: 1900–1908.

Ragot S, Marquet P, Lachatre F, Rousseau A, Lacassie E, Gaulier JM, Dupuy JL and Lachatre G. Sensitive determination of irinotecan (CPT-11) and its active metabolite SN-38 in human serum using liquid chromatography–electrospray mass spectrometry. *Journal of Chromatography B*, 1999; **736**: 175–184.

Redinbo MR, Stewart L, Kuhn P, Champoux JJ and Hol WG. Crystal structures of human topoisomerase I in covalent and noncovalent complexes with DNA. *Science* 1998; **279**: 1504–1513.

Rivory LP and Robert J. Reversed-phase high-performance liquid chromatographic method for the simultaneous quantitation of the carboxylate and lactone forms of the camptothecin derivative irinotecan, CPT-11, and its metabolite SN-38 in plasma. *Journal of Chromatography B* 1994; **661**: 133–141.

Rivory LP, Chatelut E, Canal P, Mathieu-Boue A and Robert J. Kinetics of the *in vivo* interconversion. *Cancer Research* 1994; **54**: 6330–6333.

Rivory LP, Findlay M, Clarke S and Bishop J. Reversed-phase high-performance liquid chromatographic method for the simultaneous quantitation of the carboxylate and lactone forms of the camptothecin derivative irinotecan, CPT-11, and its metabolite SN-38 in plasma. *Journal of Chromatography B* 1998; **714**: 355–359.

Rivory LP, Riou JF, Haaz MC, Sable S, Vuilhorgne M, Commerçon A, Pond SM and Robert J. Identification and properties of a major plasma metabolite of irinotecan (CPT-11) isolated from the plasma of patients. *Cancer Research* 1996; **56**: 3689–3694.

Rouits E, Charasson V, Pétain A, Boisdron-Celle M, Delord JP, Fonck M, Laurand A, Poirier AL, Morel A, Chatelut E, Robert J and Gamelin E. Pharmacokinetic and pharmacogenetic determinants of the activity and toxicity of irinotecan in metastatic colorectal cancer patients. *British Journal of Cancer* 2008; **99**: 1239–1245.

Sai K, Kaniwa N, Ozawa S and Sawada J. A new metabolite of irinotecan whose formation is mediated by human hepatic cytochrome P-450 3A4. *Drug Metabolism and*. *Disposition* 2001; **29**: 1505–1513.

Sai K, Kaniwa N, Ozawa S and Sawada J. An analytical method for irinotecan (CPT- 11) and its metabolites using a high-performance liquid chromatography: parallel detection with fluorescence and mass spectrometry. *Biomedical Chromatography* 2002; **16**: 209–218.

Sano K, Yoshikawa M, Hayasaka S, Satake K, Ikegami Y, Yoshida H, Ishikawa T, Sawada S and Tanabe S. Simple non-ion-paired high-performance liquid chromatographic method for simultaneous quantitation of carboxylate and lactone forms of 14 new camptothecin derivatives. *Journal of Chromatography B* 2003; **795**: 25–34.

Santos A, Zanetta S, Cresteil T, Deroussent A, Pein F, Raymond E, Vernillet L, Risse ML, Boige V, Gouyette A and Vassal G. Metabolism of irinotecan (CPT-11) by CYP3A4 and CYP3A5 in humans. *Clinical Cancer Research* 2000; **6**: 2012–2020.

Schoemaker NE, Rosing H, Jansen S, Schellens JH and Beijnen JH. High-performance liquid chromatographic analysis of the anticancer drug irinotecan (CPT-11) and its active metabolite SN-38 in human plasma. *Therapeutic Drug Monitoring* 2003; **25**: 120–124.

Shimada Y, Yoshino M, Wakui A, Nakao I, Futatsuki K, Sakata Y, Kambe M, Taguchi T and Ogawa N. Phase II study of CPT-11, a new camptothecin derivative, in metastatic colorectal cancer. CPT-11 Gastrointestinal Cancer Study Group. *Journal of Clinical Oncology* 1993; **11**: 909–913.

Slatter JG, Schaaf LJ, Sams JP, Feenstra KL, Johnson MG, Bombardt PA, Cathcart KS, Verburg MT, Pearson LK, Compton LD, Miller LL, Baker DS, Pesheck CV and Lord RS 3rd. Pharmacokinetics, metabolism, and excretion of irinotecan (CPT-11) following I.V. infusion of [(14)C] CPT-11 in cancer patients. *Drug Metabolism and Disposition* 2000; **28**: 423–433.

Slichenmyer WJ, Rowinsky EK, Donehower RC and Kaufmann SH. The current status of camptothecin analogues as antitumor agents. *Journal of the National Cancer Institute* 1993; **85**: 271–291.

Sparreboom A, de Jonge MJ, de Bruijn P, Brouwer E, Nooter K, Loos WJ, van Alphen RJ, Mathijssen RH, Stoter G and Verweij J. Irinotecan (CPT-11) metabolism and disposition in cancer patients. *Clinical Cancer Research* 1998a; **4**: 2747–2754.

Sparreboom A, de Bruijn P, de Jonge MJ, Loos WJ, Stoter G, Verweij J and Nooter K. Liquid chromatographic determination of irinotecan and three major metabolites in human plasma, urine and feces. *Journal of Chromatography B* 1998b; **712**: 225–235.

Srinivas NR. Dodging matrix effects in liquid chromatography tandem mass spectrometric assays—compilation of key learnings and perspectives. *Biomedical Chromatography* 2009a; **23**: 451–454.

Srinivas NR. Sensitivity enhancement in tandem liquid chromatographic mass spectrometric assays by summation of two transition ion pairs—perspectives. *Journal of Separation Sciences* 2009b; **32**: 483–486.

Stephens RH, O'Neill CA, Warhurst A, Carlson GL, Rowland M and Warhurst G. Kinetic profiling of P-glycoprotein-mediated drug efflux in rat and human intestinal epithelia. *Journal of Pharmacology and Experimental Therapeutics* 2001; **296**: 584–591.

Stewart L, Redinbo MR, Qiu X, Hol WG and Champoux JJ. A model for the mechanism of human topoisomerase I. *Science* 1998; **279**: 1534–1541.

Sugiyama Y, Kato Y and Chu X. Multiplicity of biliary excretion mechanisms for the camptothecin derivative irinotecan (CPT-11), its metabolite SN-38, and its glucuronide: role of canalicular multispecific organic anion transporter and P-glycoprotein. *Cancer Chemotherapy and*. *Pharmacology* 1998; **42**(suppl.): S44–49.

Sumiyoshi H, Fujiwara Y, Ohune T, Yamaoka N, Tamura K and Yamakido M. High- performance liquid chromatographic determination of irinotecan (CPT-11) and its active metabolite (SN-38) in human plasma. *Journal of Chromatography B* 1995; **670**: 309–316.

Thompson PA, Gupta M, Rosner GL, Yu A, Barrett J, Bomgaars L, Bernstein ML, Blaney SM and Mondick J. Pharmacokinetics of irinotecan and its metabolites in pediatric cancer patients: a report from the children's oncology group. *Cancer Chemotherapy and Pharmacology* 2008; **62**: 1027–1037.

Xuan T, Zhang JA and Ahmad I. HPLC method for determination of SN-38 content and SN-38 entrapment efficiency in a novel liposome-based formulation, LE-SN38. *Journal of Pharmaceutical Biomedical Analysis* 2006; **41**: 582–588.

Yamamoto N, Tamura T, Murakami H, Shimoyama T, Nokihara H, Ueda Y, Sekine I, Kunitoh H, Ohe Y, Kodama T, Shimizu M, Nishio K, Ishizuka N and Saijo N. Randomized pharmacokinetic and pharmacodynamic study of docetaxel: dosing based on body-surface area compared with individualized dosing based on cytochrome P450 activity estimated using a urinary metabolite of exogenous cortisol. *Journal of Clinical Oncology* 2005; **23**: 1061–1069.

Yamazaki M, Neway WE, Ohe T, Chen I, Rowe JF, Hochman JH, Chiba M and Lin JH. In vitro substrate identification studies for p-glycoprotein-mediated transport: species difference and predictability of *in vivo* results. *Journal of Pharmacology and Experimental Therapeutics* 2001; **296**: 723–735.

Yang X, Hu Z, Chan SY, Goh BC, Duan W, Chan E and Zhou S. Simultaneous determination of the lactone and carboxylate forms of irinotecan (CPT-11) and its active metabolite SN-38 by high-performance liquid chromatography: application to plasma pharmacokinetic studies in the rat. *Journal of Chromatography B* 2005; **821**: 221–228.

