Sensitive HPLC-Fluorescence Method for Irinotecan and Four Major Metabolites in Human Plasma and Saliva: Application to Pharmacokinetic Studies

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Background: We developed gradient HPLC methods for quantification of the antimitotic drug irinotecan (CPT-11) and its four metabolites, SN-38, SN-38 G, 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]-carboxyloxycampothecin (APC), and 7-ethyl-10-[4amino-1-piperidino]-carboxyloxycampothecin (NPC), as the sum of the lactone and carboxylate forms, in human plasma and saliva. Camptothecin was used as internal standard.

Methods: The sample pretreatment involved protein precipitation with methanol–acetonitrile (50:50 by volume) followed by acidification with hydrochloric acid to convert the lactone ring-opened form into its lactone form, quantitatively. HPLC separation was performed on an Xterra RP18 column. The excitation wavelength was 370 nm, and the emission wavelength was set at 470 nm for the first 24 min and then at 534 nm for the next 4 min. The stabilities of irinotecan and its four metabolites in plasma, saliva, and acidic extracts were also investigated under various conditions.

Results: Assays were linear in the tested range of 0.5–1000 μg/L. For the five analytes, limits of quantification were 0.5 μg/L in both matrices. The interassay imprecision (as relative standard deviation) was 3.2–14% in plasma and 2.6–5.6% in saliva. Assay recoveries ranged from 92.8% to 111.2% for plasma and 100.1% to 104.1% for saliva. Mean extraction recovery from plasma or saliva was 90%.

Conclusion: The developed assay can be used to determine pharmacokinetic parameters for CPT-11, SN-38, SN-38 G, APC, and NPC in plasma and saliva from patients with metastatic colorectal cancer.

The antineoplastic agent irinotecan hydrochloride (7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carboxyloxy-camptothecin; CPT-11; Fig. 1) is a semisynthetic derivative of the natural product camptothecin (1, 2). This drug has demonstrated good antitumor activity both in vitro and in vivo against various experimental tumor models (1, 3), including multidrug-resistant lines (4). CPT-11 has shown anticancer activity against a variety of solid tumors in clinical trials, including colorectal cancer, gynecologic cancers, non-small cell and small cell lung cancers, and refractory cervical cancer (5–11). Like camptothecin, CPT-11 acts by inhibiting mammalian DNA topoisomerase I (12, 13). Cell death results from stabilization of cleavable complexes formed between topoisomerase I and DNA during DNA replication, transcription, and repair (14–17).

CPT-11 is extensively metabolized in the liver into various metabolites. It is enzymatically cleaved by carboxylesterases to form 7-ethyl-10-hydroxycampothecin (SN-38), which has a cytotoxic activity 100 to 1000 times greater than that of the parent drug (18–20). SN-38 is subsequently conjugated by uridine diphosphate glucuronosyltransferases to an inactive β-glucuronide derivative (SN-38G) (20–22). Other known human metabolites of CPT-11 include 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]-carboxyloxycampothecin (APC)4 and a

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4 Nonstandard abbreviations: APC, 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]-carboxyloxycampothecin; NPC, 7-ethyl-10-[4-amino-1-piperidino]-carboxyloxycampothecin; QC, quality-control; tr, retention time; RSD, relative standard deviation; and AUC, area under the curve.
primary amine metabolite, 7-ethyl-10-[4-amino-1-piperidino]-carbonyloxycamptothecin (NPC), which results from ring-opening oxidation of the terminal piperidine ring of CPT-11 mediated by cytochrome P450 3A4 enzymes (23, 24). APC and NPC have weak inhibitory activity against cell growth in vivo. The structures of these metabolites are presented in Fig. 1.

CPT-11 and its metabolites contain an α-hydroxy-δ-lactone ring, which is chemically unstable and undergoes pH-dependent reversible hydrolysis to a hydroxyl-carboxylate form. Only the closed lactone forms of the drug inhibit topoisomerase I. Rivory et al. (25) found low variability of this interconversion despite the fact that the patients received different doses and were at different stages of treatment.

Several HPLC methods have been developed to quantify irinotecan and its major metabolites in human plasma (26–37). These methods involve the simultaneous quantification of the lactone and carboxylate forms (27, 29, 32, 37) or quantification of the total forms, i.e., lactone plus carboxylate (26, 28, 30, 31, 33–36). In most of these methods, both CPT-11 and SN-38 were quantified and the related compound camptothecin was used as the internal standard. Only Sparreboom et al. (30), Sai et al. (35), and Owens et al. (37) developed a method for the determination of CPT-11 and the three metabolites SN-38, SN-38G, and APC in biological matrices. In most of these published methods, chromatography was carried out with fluorescence detection. Liquid chromatography–mass spectrometry methods have also been described (33, 35), but despite the latter methods providing good results, the instrumentation involved is expensive and not always readily available for routine drug monitoring.

Takahashi et al. (38) found that both CPT-11 and SN-38 were detectable in saliva and that the patterns of their concentration–time curves in plasma and saliva were very similar. To date, however, no bioanalytical assay has been validated for the determination of irinotecan and its metabolites in saliva.

This report describes rapid, specific, reliable, and sensitive analytical methods to simultaneously quantify irinotecan and four metabolites (SN-38, SN-38G, APC, and NPC) in human plasma and saliva. These methods, involving the use of an internal standard, were validated according to validation procedures, parameters, and acceptance criteria based on US Pharmacopoeia XXIII guidelines and Food and Drug Administration guidance (39–42). We also evaluated the stability of these analytes.
under various conditions. The suitability of the developed methods for clinical use was demonstrated by the determination of irinotecan and its four metabolites in plasma and saliva obtained from patients with metastatic colorectal cancer.

**Materials and Methods**

**MATERIALS AND REAGENTS**

Irinotecan (hydrochloride salt; CPT-11), SN-38, SN-38 G, APC, and NPC were kindly donated by Aventis Pharma Laboratories (Vitry-sur-Seine, France). The internal standard, camptothecin, was obtained from Sigma. Stock solutions of each compound were prepared in dimethyl sulfoxide (Carlo Erba) at the concentration of 1 g/L each and stored at −80 °C. Methanol, dimethyl sulfoxide, acetone, acetonitrile, 5 mol/L HCl, monopotassium phosphate, orthophosphoric acid (all from Merck), and 1-heptanesulfonic acid (Sigma) were all of analytical-reagent grade. The 5 mol/L HCl was diluted fivefold in sterile water (Fresenius, France Pharma). The phosphate buffer consisted of 13.609 g of monopotassium phosphate and 0.607 g of 1-heptanesulfonic acid in 1 L of sterile water adjusted to pH 4.0 with orthophosphoric acid.

For method validation, human plasma (Etablissement Français du sang) and parotid saliva were obtained from pooled samples collected from healthy volunteers. Saliva and plasma were stored at −20 °C before use.

**INSTRUMENTATION**

The chromatographic system consisted of two Shimadzu LC-10AT pumps and a mixing chamber, a Shimadzu Model RF-10Axl fluorescence detector, an automatic sample injection system (Model 717; Waters) thermostatted at 10 °C, and a Shimadzu data acquisition station (Class VP 4.2). The excitation wavelength was 370 nm; the emission wavelength was set at 470 nm for the first 24 min and at 390 nm for the next 4 min. HPLC separation was performed in a stainless-steel Xterra RP18 column [20 × 3.9 mm (i.d.); 5-μm particle size; Waters] placed just before the inlet of the analytical column to reduce contamination.

**CHROMATOGRAPHIC CONDITIONS**

The eluent was pumped through the column at a flow rate of 1 mL/min and consisted of a mixture of acetonitrile–sterile water (75:25 by volume; solvent A) and phosphate buffer, pH 4 (solvent B). The mobile phases were filtered through a 0.45 μm HV filter (Millipore), then degassed ultrasonically before use. Table 1 shows the various proportions of solvents A and B used for the methods. Chromatography was performed at ambient temperature (23 °C).

**SAMPLE PREPARATION**

To a polypropylene microtube containing 100 μL of an internal standard solution (100 μg/L in acetone), previously dried under nitrogen stream, we added 0.5 mL of plasma (or 0.1 mL of saliva) and a mixture of methanol–acetonitrile (50:50 by volume; 1 mL for plasma or 0.2 mL for saliva). Proteins were denatured by full-speed vortex-mixing for 15 s; samples were then centrifuged at 9500g for 4 min. The clear supernatant (550 μL for plasma or 220 μL for saliva) was aspirated and placed in another microtube to which 250 μL (or 100 μL) of 1 mol/L HCl was added. Samples were vortex-mixed for 15 s, and an aliquot of this solution (10 μL at high concentrations or 100 μL at low concentrations) was injected on the column.

Calibrators (0.5, 1, 2.5, 5, 10, 25, 50, 100, 250, 500, and 1000 μg/L) were prepared by mixing drug-free plasma or saliva with appropriate volumes of working solutions in acetonitrile–purified water (50:50 by volume). The sample pretreatment procedure was identical to that described above.

**DATA ANALYSIS**

For each calibration curve (0.5–10, 10–250, and 250-1000 μg/L), we applied an unweighted least-squares linear regression of the responses (peak-area ratios of compound to internal standard) as a function of the nominal concentrations. The detector was set at a sensitivity of 2 and a gain of 2. The parameters of each calibration curve were used to compute back-calculated concentrations and to obtain concentration values for that day’s quality-control (QC) samples and unknown samples.

The “lack-of-fit” test was used to confirm the linearity of the method. In addition, the back-calculated concentrations were compared with the nominal concentrations, and the mean differences (or mean predictor error) with the 95% confidence interval were computed.

**SELECTIVITY**

We investigated the specificity of the method by analyzing 10 different batches of blank human plasma and saliva samples from healthy volunteers to determine whether endogenous constituents coeluted with the different analytes. The retention times (t_r) of endogenous compounds in the matrix were compared with those of the compounds of interest.

Plasma and saliva samples from patients receiving other drugs were analyzed for interference. The following

**Table 1. Proportions of solvent A and solvent B for the HPLC method.**

<table>
<thead>
<tr>
<th>Time, min</th>
<th>% Solvent A</th>
<th>% Solvent B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>27.3</td>
<td>72.7</td>
</tr>
<tr>
<td>6</td>
<td>27.3</td>
<td>72.7</td>
</tr>
<tr>
<td>7</td>
<td>34.0</td>
<td>66.0</td>
</tr>
<tr>
<td>23.5</td>
<td>34.0</td>
<td>66.0</td>
</tr>
<tr>
<td>24</td>
<td>36.7</td>
<td>63.3</td>
</tr>
<tr>
<td>29</td>
<td>36.7</td>
<td>63.3</td>
</tr>
<tr>
<td>30.5</td>
<td>27.3</td>
<td>72.7</td>
</tr>
</tbody>
</table>
drugs were checked: granisetron, ondansetron, tropisetron, dolasetron, alizapride, zolpidem, tianeptine, methylprednisolone, fluoxetine, dextropropoxyphene, acetaminophen, enoxaparin, nifedipine, bromazepam, lorazepam, chlorazepate, levothyroxine, zopiclone, tinzaparín, cetirizine, simvastatin, famotidine, glibenclamide, and omeprazole.

IMPRECISION AND RECOVERY
We assessed the within- and between-day imprecision and the recoveries by performing replicate analyses of QC samples (0.75, 75, and 750 μg/L) in plasma or saliva and comparing them against the calibration curves. The procedure was repeated on different days with the same calibrators to determine between-day values. Intraday values were determined by treating samples to which calibrators had been added in replicate on the same day.

The mean recovery was calculated as (mean measured concentration/theoretical concentration) × 100. Imprecision is given as the relative standard deviation (RSD).

EXTRACTION EFFICIENCY
We determined the recoveries of CPT-11, SN-38, SN-38G, APC, and NPC from plasma and saliva twice at three concentrations (0.75, 75 and 750 μg/L) by calculating the percentage difference between the peak areas of extracted calibrators and those of the authentic (unextracted) calibrators in the relevant concentration ranges prepared in acetonitrile–water–1 mol/L HCl (183:367:250 by volume). The extraction recovery was also computed for the internal standard.

DETERMINATION OF THE LOWER LIMIT OF QUANTIFICATION
The lower limit of quantification, estimated from QC samples, was defined as the lowest drug concentration that could be determined with a RSD <20% and a recovery of 100 (20)% on a day-to-day basis (39–42).

STABILITY STUDY
The stability of the lactone plus carboxylate forms of irinotecan and its four metabolites in plasma and saliva was determined by use of QC samples at concentrations of 0.75, 75, and 750 μg/L as follows:

(a) By storing QC samples at room temperature with daylight exposure (~23 °C) and in a refrigerator at 4 °C for 24 h.
(b) By storing QC samples in plasma and saliva at −20 °C for 6 and 4 months, respectively. Before their analysis, samples were brought to room temperature and vortex-mixed well.
(c) By subjecting aliquots of plasma and saliva to three freeze-thaw cycles. Frozen samples were allowed to thaw at ambient temperature for 1 h and were subsequently refrozen.
(d) By storing the acidic solution (pH 1) originating from plasma to which irinotecan and its metabolites had been added in the autosampler at 23, 10, and 4 °C for 48, 72, and 72 h, respectively.

QC samples were analyzed immediately after preparation (reference values) and at selected time intervals after storage over the study period. Three replicates were analyzed at each time point. Stability was defined as <10% loss of initial drug concentration.

PHARMACOKINETIC STUDY
The pharmacokinetic study was carried out in patients with metastatic colorectal cancer. It was conducted according to the current revision of the Helsinki Declaration, and all patients gave informed consent. Irinotecan was administered in a 90-min infusion every 2 weeks (180 mg/m² for the first course, 220 mg/m² for the second course, and 260 mg/m² for the following courses). The patients had satisfactory WHO Cooperative Oncology Group scores (0 or 2) and liver and kidney functions. Special attention was paid to sample handling. Blood collected in heparin-containing tubes and unstimulated saliva samples were drawn before drug administration (t₀) and at 30, 60, and 90 min and 4, 8, 12, 24, and 42 h after the start of infusion. Blood samples were centrifuged (1500g for 10 min) at 4 °C. A 1-mL aliquot of plasma was transferred to another tube. Saliva samples were centrifuged at 9500g for 4 min before storage. Plasma and saliva samples were stored at −80 °C until assay. To quantify the different compounds in plasma and saliva samples, we ran calibration curves and three QC samples with every set of 20 unknown samples. For CPT-11 concentrations higher than the highest calibration point, we diluted samples with drug-free plasma to bring the concentrations within the range of calibration curve. Pharmacokinetic parameters were calculated using Pk-fit software (43). The areas under the plasma or saliva concentration–time curves (AUC) from time zero to infinity were obtained by linear trapezoidal approximation with correction to time infinity by dividing the last observed data point by the elimination rate constant. Elimination half-life was determined from the slope of the linear part of the semilogarithmic curves.

**Results and Discussion**

RETENTION TIMES AND SPECIFICITY
Representative chromatograms of drug-free human plasma and saliva; plasma and saliva with added CPT-11, SN-38, SN-38G, APC, NPC, and internal standard; and postdose human plasma and saliva samples collected from the clinical study are shown in Figs. 2 and 3, respectively. Under the chromatographic conditions used, the number of theoretical plates (computed on the peak of internal standard) was ~12 531. The precolumn was exchanged every 200 sample runs, and the column was replaced when the number of theoretical plates had decreased to <7604. SN-38G [mean (SD) tᵣ = 5.54 (0.15)
min; n = 40], NPC \([t_r = 13.9 (0.23) \text{ min}; n = 40]\), APC \([t_r = 16.0 (0.36) \text{ min}; n = 40]\), CPT-11 \([t_r = 19.6 (0.56) \text{ min}; n = 40]\), internal standard \([t_r = 22.5 (0.54) \text{ min}; n = 40]\), and SN-38 \([t_r = 28.2 (0.83) \text{ min}; n = 40]\) exhibited well-separated peaks \((\alpha_{1,2} = 1.4, \alpha_{2,3} = 1.3, \alpha_{3,4} = 1.4, \alpha_{4,5} = 1.3, \alpha_{5,6} = 1.5)\) under the chromatographic conditions described. The \(k'\) values were 0.89, 3.56, 4.27, 5.43, 6.36, and 8.3 for the six analytes, respectively. We evaluated peak skew using the asymmetry coefficient \(A_s = b/a\), where \(b\) is the distance after the peak maximum and \(a\) is the distance before the peak maximum, both \(a\) and \(b\) being measured at 10% of the total peak height. The asymmetry coefficients were \(1.09\) for SN-38G, \(1.03\) for NPC, \(1.23\) for APC, \(1.27\) for CPT-11, and \(0.9\) for SN-38. No endogenous interfering peaks were visible at the retention times of the different analytes (Figs. 2A and 3A). No interference was found with all tested drugs that could be coadministered.

LINEARITY
The calculated peak-area ratios and the added concentrations of each analyte displayed linear relationships over the selected concentration ranges with consistent slopes and coefficients of determination \((r^2) > 0.99\) throughout the validation runs. The RSD for the slopes of calibration curves prepared on the same day or on different days was \(< 13\%\) in plasma and \(< 9.5\%\) in saliva. The lack-of-fit test showed no significant deviation from linearity. For each calibration point, the concentrations were back-calculated from the equation of the linear regression curves. Linear regression of the back-calculated concentrations vs the nominal concentrations provided a unit slope and an intercept equal to 0 (Student \(t\)-test). The distribution of the residuals (difference between nominal and back-calculated concentrations) showed random variations, the number of positive and negative values being approximately equal. Moreover, they were normally distributed and centered around zero. The mean value of residuals was not statistically different from 0 (Student \(t\)-test), and the 95% confidence interval included the 0 value.

IMPRECISION AND RECOVERY
The recovery and imprecision results are shown in Table 2. The mean (SD) extraction efficiencies for CPT-11, SN-38, SN-38G, APC, and NPC from human plasma were 95.5 (5.4)%; 97.2 (5.9)%; 93.4 (5.0)%; 95.5 (5.8)%; and 99.2 (4.69)%; respectively \((n = 6)\). In saliva, they were 97.5
(4.2)%, 96.8 (9.5)%, 94.6 (8.2)%, 103.9 (6.3)%, and 100.8 (7.4)%, respectively (n = 6). Recoveries for the internal standard were 99.5 (3.0)% (n = 3) in plasma and 99.2 (5.0)% (n = 3) in saliva.

The extraction efficiency was independent of concentration over the range studied.

Table 2. Recovery and imprecision of the method.

<table>
<thead>
<tr>
<th>Theoretical concentration, µg/L</th>
<th>Imprecision, %</th>
<th>Recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SN-38G NPC APC CPT-11 SN-38</td>
<td>SN-38G NPC APC CPT-11 SN-38</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within-day (n = 6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.75</td>
<td>5.1 14 10 8.0 9.3</td>
<td>104.1 103.4 95.7 102.6 102.8</td>
</tr>
<tr>
<td>75</td>
<td>6.2 7.4 1.0 2.6 2.2</td>
<td>98.6 104.5 90.0 100.4 102.9</td>
</tr>
<tr>
<td>750</td>
<td>4.4 4.0 4.3 5.0 4.3</td>
<td>99.3 97.3 101.0 98.9 98.7</td>
</tr>
<tr>
<td>Between-day (n = 7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.75</td>
<td>5.9 14 11 9.9 3.6</td>
<td>102.4 101.6 100.2 102.6 111.2</td>
</tr>
<tr>
<td>75</td>
<td>3.2 3.7 3.8 3.5 3.6</td>
<td>92.8 95.2 97.3 95.6 96.6</td>
</tr>
<tr>
<td>750</td>
<td>4.6 4.7 5.1 4.6 4.5</td>
<td>95.3 96.1 95.9 96.4 97.4</td>
</tr>
<tr>
<td>Saliva</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between-day (n = 7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.75</td>
<td>4.5 3.7 2.6 4.9 5.3</td>
<td>100.1 104.1 101.7 101.3 103.5</td>
</tr>
<tr>
<td>75</td>
<td>5.4 5.6 5.2 5.1 3.8</td>
<td>102.1 101.6 102.1 101.4 101.4</td>
</tr>
<tr>
<td>750</td>
<td>4.2 4.3 4.3 4.4 3.8</td>
<td>101.1 100.7 101.4 100.3 100.1</td>
</tr>
</tbody>
</table>

LOWER LIMIT OF QUANTIFICATION

In plasma and saliva, the lower limit of quantification was 0.5 µg/L for irinotecan and its four metabolites. For QC samples, the RSD did not exceed 20%, and recoveries were 83–108%. Compared with the published methods for most of the analytes, this limit of quantification was
better than those published in the literature (26–30, 33–37).

STABILITY
Frozen QC samples tested over a 4-month period showed no sign of either degradation or loss in both plasma and saliva (P >0.05). We performed stability assays on frozen plasma samples over a 6-month period. We observed significant degradation, averaging 37% for SN-38G at 0.75 µg/L, but observed no degradation for SN-38G at the other concentrations of any of the other compounds at any of the concentrations studied.

Plasma and saliva QC samples with added CPT-11, SN-38, SN-38G, APC, and NPC allowed to stand at room temperature or at 4 °C for 24 h showed no sign of decrease in the nominal starting concentration.

In acidic extracts, APC showed a substantial decrease after 24 h of storage at ambient temperature; mean (SD) losses were 49.1 (2.1)% at 0.75 µg/L, 45.8 (2.5)% at 75 µg/L, and 38.9 (1.9)% at 750 µg/L. For this compound, the decrease in drug concentration followed a monoeponential pattern; the corresponding half-life value was 21 h.

Fig. 4 shows the stability at ambient temperature of the different analytes (concentration, 75 µg/L) in acidic extracts for the first 16 h. At 10 °C, losses were <10% after 8 h of storage, whereas after 24 h, mean losses were 9.1 (5.2)%), 21.7 (4.1)%), and 17.6 (2.8)% at the three concentrations, respectively. Losses were lower at 4 °C [7.8 (0.5)%), 18.6 (1.0)%), and 11.1 (2.0)%), respectively, after 72 h of storage]. NPC concentrations increased in proportion to the decrease in APC (30–44% increase after 24 h at ambient temperature), suggesting a partial conversion of APC into NPC in acidic extracts. The stability of SN-38G in acidic extracts indicated that no statistically significant degradation occurred over a span of 48 h at ambient temperature at 0.75 µg/L [recovery, 106 (8.1)%]. For the two other concentrations tested, we observed a significant decrease after 24 h [mean recovery, 80.3 (1.4)% at 75 µg/L and 81.8 (4.0)% at 750 µg/L]. At 4 and 10 °C, SN-38G was stable for 72 h. CPT-11, SN-38, and the internal standard were stable in the different tested conditions.

Interestingly, both plasma and saliva samples showed no sign of degradation after the freeze–thaw procedure.

These stability results are of great interest for the routine analysis of a large number of samples during pharmacokinetic studies. Thus, acidic extracts from biological samples must be kept at low temperatures before and during analysis to avoid rapid degradation.

PHARMACOKINETIC STUDY
The plasma and saliva concentration–time profiles of CPT-11 and the observed metabolites determined by the method described above are presented in Fig. 5. These results were obtained from the first three patients included in the pharmacokinetic study (administered dose, 180 mg/m²).

Fig. 5. Mean (SD; error bars) plasma (A) and saliva (B) concentration–time profiles for irinotecan and its four metabolites from the first three patients included in the pharmacokinetic study (administered dose, 180 mg/m²). ×, SN-38G; ◆, NPC; △, AP and □, APC; •, SN-38; ⌓, CPT-11; ■ and □, SN-38.
Table 3. Main pharmacokinetic parameters (mean (SD)) for irinotecan and its metabolites computed from results for the first three patients included in the study.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Plasma</th>
<th>Saliva</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>SN-38G</td>
<td>1.5 (1.4)</td>
<td>122 (39.6)</td>
<td>0.29</td>
</tr>
<tr>
<td>NPC</td>
<td>3.2 (1.4)</td>
<td>17.0 (6.4)</td>
<td>0.40</td>
</tr>
<tr>
<td>APC</td>
<td>2.8 (1.4)</td>
<td>5.4 (4.1)</td>
<td>0.98</td>
</tr>
<tr>
<td>CPT-11</td>
<td>3.2 (1.4)</td>
<td>194 (40.8)</td>
<td>0.15</td>
</tr>
<tr>
<td>SN-38</td>
<td>2.16 (1.6)</td>
<td>39.7 (17.9)</td>
<td>0.98</td>
</tr>
</tbody>
</table>

a $t_{\text{max}}$, time to peak; $c_{\text{max}}$, peak plasma concentration.
b $\text{AUC}_{\text{saliva}}/\text{AUC}_{\text{plasma}}$.

tial pattern. The main pharmacokinetic parameters are given in Table 3. From the plasma data, the CPT-11 total clearance was 15.6 (4.0) L · h$^{-1}$ · m$^{-2}$. Rapid diffusion of irinotecan in saliva occurred; its concentrations were of the same order of magnitude in saliva and plasma samples, and the saliva/plasma AUC ratio averaged 0.98. For the active metabolite SN-38, concentrations in saliva were approximately twofold lower than in plasma; the saliva/plasma AUC ratio was 0.40. For the two metabolites APC and NPC, diffusion in saliva was low, whereas SN-38G concentrations in saliva were lower than the lower limit of quantification of the method. The low diffusion of these three metabolites could be explained by their high hydrophilic properties. Considering the high binding rates of irinotecan lactone in saliva, the secretion of these two compounds in saliva remains important. The carboxylate is the predominant form of irinotecan in plasma soon after the end of infusion (25); because this form is unlikely to cross biological barriers, the secretory mechanisms of the irinotecan lactone in saliva are not attributable to simple passive diffusion but rather to active processes, as reported recently for topotecan (44). Thus, the saliva concentration could be a good predictor of the behavior of irinotecan in the body.

In conclusion, this fully validated method permits simple and rapid quantification of irinotecan and its four main metabolites in plasma and saliva samples. In many clinical situations, including patients with difficult venous access, the determination of irinotecan and its active metabolite SN-38 in saliva may be relevant.

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References
