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Review Article

IRINOTECAN-REVIEW OF ANALYTICAL METHODS DEVELOPED FOR PHARMACEUTICAL DOSAGE FORMS AND BIOLOGICAL FLUIDS

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ABSTRACT

Irinotecan (IRI) is utilised as a first line anticancer medication in the cure of cancer having extraordinary ability to block DNA-topoisomerase-I. It is used as a monotherapy and adjunct therapy in the treatment of metastatic colorectal cancer and other cancers, and it differs chemically and pharmacologically from other anticancer medications. The proposed review is divided into two main sections i.e. a) Different analytical methods for estimating irinotecan in pharmaceutical formulations, b) Diverse analytical methods for detecting irinotecan in biological matrices. This work also considers the development of numerous analytical methods based on various parameters, as well as the validation of the methods used. Estimated validation characteristics such as Linearity, Limit of Detection (LOD), and Limit of Quantitation (LOQ) are considered for each. Applying bioanalytical methods, the wavelength of detection, mobile phase, columns, flow rate, retention duration, and sample preparation processes are all evaluated as essential quality variables for estimating Irinotecan.

Keywords: Irinotecan, Analytical methods, Biological matrices

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INTRODUCTION

Irinotecan is a cytotoxic medication used as 1st line therapy in conjunction with 5-fluorouracil for the treatment of colorectal cancer that has spread to other parts of the body [1, 2]. CPT-11 and Camptothecin-11 are other names for irinotecan. Camptothecin, a cytotoxic alkaloid found in plants like Camptotheca acuminata, is a water-soluble semisynthetic derivative. Irinotecan and other camptothecin analogues/derivatives have demonstrated anticancer activities in *in vitro* as well as *in vivo* against a number of experimental tumour forms, including multidrug-resistant cell lines [2, 3]. Irinotecan was approved for cancer treatment in Japan for the first time around 25 y ago. IRI has made important contributions to the treatment of solid tumours all around the world for more than two decades [4]. It is a therapy for colorectal and small cell lung cancer that has been approved by the United States–Food and Drug Act (US-FDA) [3, 5]. Chemically, IRI is a Camptothecin analogue, available with trade names CAMPTOSAR, IRINOMIL, IRNOCAM, TORSIRIN, IRINOTEL, IRBEST [1-6]. Its molecular formula is $C_{33}H_{38}N_4O_6$, molecular weight is 586.7 g/mol, available in solid state as pale yellow color powder. Its melting point is 222 °C. It is hygroscopic, soluble in water with a water solubility of 0.107 g/l. It is also reported to be light-sensitive compound [6]. IRI inhibits DNA-topoisomerase-I and causes cell death by stabilising the complexes generated during DNA replication. It is a pro-drug that is hydrolysed by enzymes to produce a metabolite that is active (7-ethyl-10-hydroxycamptothecin, SN-38). Both of these molecules exist in two states at the same time: a lactone form that is active and a carboxylate form that is inactive with no inhibitory activity against topoisomerase I. At lower pH, lactone forms predominate, whereas carboxylate forms predominate at above pH 8 [5]. As IRI exhibits cytotoxic activity, it is employed in the cure of different types of cancers. IRI is available as an intravenous dosage form in two strengths i.e., 20 mg/ml and 4.3 mg/ml, respectively. Each patient's determined volume is taken from the vial and diluted with 500 ml of dextrose 5 percent water (D5W) or normal saline (NS) [1]. IRI's volume of distribution ranges from 110 to 234 L, with protein binding ranging from 30 to 68% [1]. It is extensively metabolised by the liver via several enzyme systems, culminating in the generation of SN-38, a potent active metabolite. The enzyme carboxylesterase breaks down the Di-piperidinocarbonyloxy group of irinotecan to create carboxylic acid and SN-38. Hepatic UDP-glucuronyl transferase can convert SN-38 to SN-38 glucuronide [7]. With a total body clearance of 13.3 to 13.9 L/h, it is eliminated via the biliary and renal routes (about 25 to 50% in 48 h). Irinotecan has a half-life of 6 to 12 hr. Because of considerable inter-individual pharmacokinetic variability, the exact therapeutic range of IRI is unknown. Alopecia, vomiting, neutropenia, asthenia, diarrhoea, fever, myelosuppression, and thrombocytopenia are among side effects of IRI [1].

Because IRI is so important in cancer treatment, rigorous quality control methods should be adopted to ensure drug purity and efficacy. First and foremost, analytical methods for certifying and quantifying pharmaceuticals in pharmaceutical formulations and biological matrices are reviewed. It becomes critical for compiling the numerous analytical methodologies established for calculating IRI.

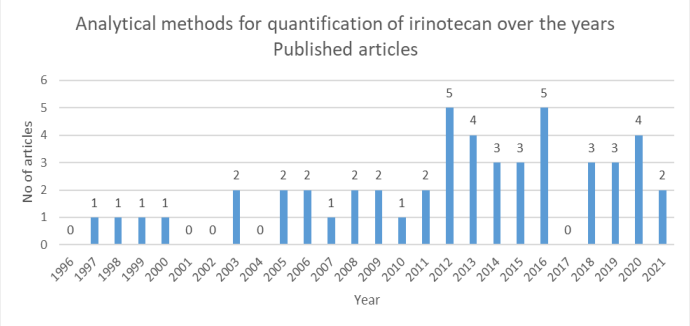

Fig. 1: Reported methods of analysis from 1996–2020

The numerous analytical approaches for calculating IRI in pharmaceutical formulations are discussed in this article. The literature was obtained from various databases i.e. Science Direct, Scopus, Taylor and Francis, Web of Sciences, Elsevier, Springer, PubMed. The data collected was from 1996-2020.

Fig. 1 shows a visual representation of key studies on analytical approaches used for IRI estimation published between 1996 and 2020. IRI has been calculated in a number of formulations and biological fluids over the years.

The graphical representation in fig. 1 is primarily intended to address various approaches that have been established as well as to provide insight into what other methods may be produced in the future. Fig. 2 depicts the position of various analytical methodologies presented for estimating IRI. This review also looks at how approaches have progressed, from ultra violet (UV) spectrophotometry through fluorimetric detection to hyphenated techniques.

Spectroscopic methods

Ultraviolet and spectrophotometric methods

UV spectrophotometry, which uses UV absorption and chemical reactions to identify and quantify IRI, is an important technology. For routine analysis, it is a low-cost, simple, quick, selective, and accurate approach. This approach is often used in laboratories, because it is versatile and cost-effective. The numerous spectrophotometry methods for detecting and estimating IRI in pharmaceutical formulations are shown in table 1 [8].

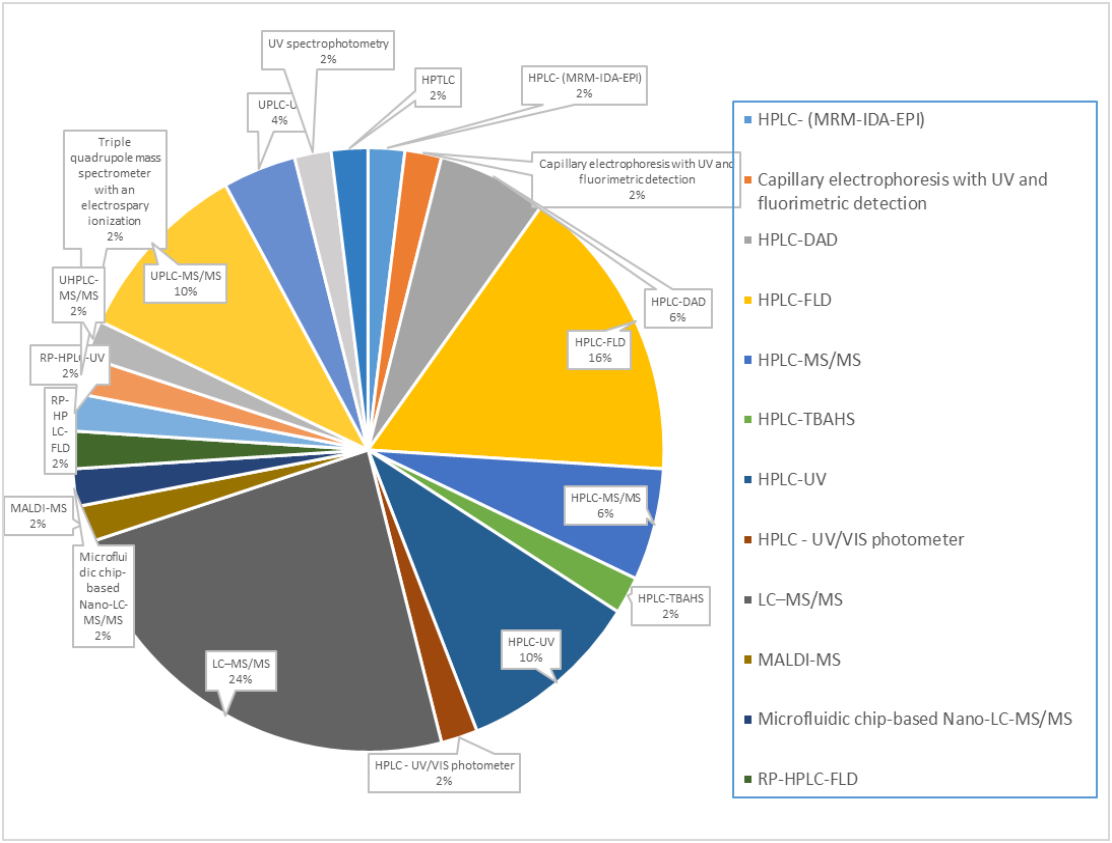

Fig. 2: An outlook of various analytical methods proposed for the estimation of IRI

Hyphenated techniques

LC-MS-MS AND UPLC-MS-MS

Liquid chromatography with tandem mass spectrometry (LC-MS-MS) is a potent analytical technology that combines liquid chromatography's separating ability with triple quadrupole mass spectrometry's sensitive and selective mass analysis capability. The anticancer drug 7-ethyl-10-hydroxycamptothecin (SN-38) has been measured intracellularly in tumour cells using an ultra-high performance liquid-chromatography–mass spectrometry-mass spectrometry (UPLC–MS-MS) approach with camptothecin (CPT) as an internal reference. The SN-38 was extracted using acidified acetonitrile (ACN). SN-38 and CPT were separated on a pentafluorophenyl (PFP) column using gradient elution with acidified water and ACN. SN-38 and CPT were measured using a triple quadrupole mass spectrometry instrument. Least-square regression calibration lines were obtained with average correlation coefficients of R^2 = 0.9993±0.0016. Under low-quality control (LQC, 5 ng/ml) and high-quality control (HQC, 500 ng/ml), SN-38 had a lower limit of detection (LOD) of 0.1 ng/ml and a lower limit of quantification (LOQ) of 0.3 ng/ml, while CPT recovery was 98.5±13 percent and SN-38 recovery was 89±6 percent and 95±8 percent, respectively [9-11].

HPLC-FLD

High-Performance Liquid Chromatography (HPLC) with fluorescence detection is one of the most widely used techniques for drug estimation since it is more reliable than other methods. In order to analyse the amount of lactone and lactone plus carboxylate (total) forms of anticancer medicine irinotecan (CPT-11) and its active metabolite SN-38 in human plasma, sensitive high-performance liquid chromatographic tests have been devised. Camptothecin, a chemical made from irinotecan, was used as an internal standard. Lactone forms were pre-treated using ACN-n-butyl chloride (1:4, %v/v) solvent extraction, whereas total forms were cleaned by aqueous perchloric acid: methanol-(1:1, %v/v) protein precipitation, resulting in carboxylate conversion to lactone forms. For chromatography, a Hypersil ODS column was employed, and fluorimetric detection was used. Lactone and total form quantification limits are 0.5 and 2.0 ng/ml, respectively [13, 14].

Table 1: Analytical methods for the estimation of IRI in analytical samples

Year	Drug	Method	Matrix	Sample preparation	Column/Mobile phase	Retention time	Detection	Linearity range	Ref.
2020 USP	IRI-HCL	HPLC	API	Dilution	Column: 4.6-mm × 25 cm; 5 µm packing L1 Flow rate: 1.5 ml/min, Mobile phase: Acetonitrile, methanol, and Solution A (17:24:59 %v/v/v), Solution A: 2.8 g/l of monobasic sodium phosphate monohydrate and 1.8 g/l of 1-octanesulfonic acid sodium salt monohydrate in water	NA	UV 255 nm	NA	[64]
2020USP	IRI-HCL	HPLC	API (RS)	Dilution	Column: 4.6 mm × 25 cm; 10 µm packing L40, Flow rate: 1.0 ml/min, Mobile phase: Hexane, dehydrated alcohol, and di-ethylamine (250:250:1)	0.55 min (for IRI related compound B), 0.60 min (for IRI related compound C), and 1.00 min (for IRI)	UV 370 nm	NA	[64]
2020USP	IRI-HCL	HPLC	Injection (Assay)	Dilution	Column: 4.6 mm × 25 cm; 5 µm packing L7 Mobile phase-Acetonitrile (ACN) and buffer (34:66 %v/v). Adjust with phosphoric acid to a pH of 2.5	NA	UV 254 nm	NA	[63]
2020USP	IRI-HCL	HPLC	Injection (RS)	Dilution	Column: 4.6 mm × 25 cm; 5 µm packing L1 Elution: Gradient, Mobile phase: Solution A: Dissolve 2 g of sodium 1 hexane sulfonate and 1 ml of triethylamine in 1 L of water. Adjust with phosphoric acid to a pH of 2.5., Solution B: Acetonitrile	0.53 min (for IRI related compound B), 0.65 min (for Camptothecin), 1.00 min (for IRI), and 1.16 min (7-Ethylcamptothecin)	UV 254 nm	NA	[63]
2021	IRI	HPLC-DAD	Standard solution	Dilution	Column: Purosher® STAR RP-18 end-capped 3 µm Hibar® RT 150-4.6 mm, Mobile phase: 20 mmol Phosphate buffer with pH 1.7: Methanol (45:55) (%v/v)	2.715 min	UV 233 nm	0.04 to 1.80 mg/ml	[18]
2018	IRI (infusion)	HPLC-DAD	Infusion solution concentrates	Dilution	Column: Zorbax Extend C18 (250 mm × 4.6 mm; 5 µm particle size); Security Guard Column (4.0 × 3.0 mm; 5 µm particle size). Mobile phase: Milli Q water and acetonitrile (96:04) (%v/v).	8.97±0.38 min	376 nm	0.1 to 18 µg/ml	[19]
2016	Tablet and parenteral formulations	UV spectrophotometry, HPTLC	Infusion solution concentrates	Dilution	Chromatography plates: Silica gel plates (Pre-Coated), Mobile phase: (Toluene: ethyl acetate: MeOH: carbon tetrachloride (CCl_4)) (9.2: 5: 0.9: 0.8) (v/v/v/v)	0.34±0.08 (R_f value)	UV 317 nm (densitometric), 247 nm (UV spectrophotometry)	200–1200 ng/spot (HPTLC), 2–10 µg/ml, (UV spectrophotometry)	[8]
2012	IRI HCl working standard injection and its impurities	UPLC-UV	Infusion solution concentrates	Dilution	Column: Waters Acquity BEH C8 column (100 × 2.1 mm, 1.7 µm particle size) Mobile phase: Solvent A-(0.02 Mol/l potassium dihydrogen ortho-phosphate, pH adjusted to 3.4 with ortho-phosphoric acid), Solvent B-(a mixture of ACN and MeOH in the ratio of 62:38 %v/v) (UPLC)	2.165 min	UV 220 nm	0.063 to 83.2 µg/ml	[26]
2021	IRI Infusion	HPLC-UV/VIS photometer	Infusion solution concentrates	Dilution	Column: ACE 5 column (Hichrom Ltd, UK) (110 Å, C18, 5 µm, 250 × 4.6 mm) (ACE 5 column, 110 Å), Mobile phase: ACN: 20 mmol phosphoric acid/sodium phosphate buffer pH 3.2 (28:72) (%v/v)	NA	220 nm (PDA)	0.90-37.00 mg/ml	[29]
2021	IRI	HPLC-UV	Injection formulation	Dilution	Column: HyperClone (Phenomenex®) C18 column (250 × 4.6 mm id, particle size 5 µm, ODS 130 Å)., Mobile phase: Acetonitrile: 20 mmol/l potassium phosphate buffer (pH 2.5) containing 0.1% triethylamine in the ratio (45:55) (%v/v).	4.09 min	UV 254 mn	0.5 to 18 µg/ml	[9]
2019	IRI	UHPLC-MS-MS	Raw material (pure Irinotecan)	Solid-phase extraction	Column: Kinetex XB-C18 column (3.0 mm × 100 mm, 1.7 µm)., Mobile phase: Solvent A-water with 0.1% formic acid., Solvent B-MeOH with 0.1% formic acid	5.96 min	Mass/Charge 587.3 → Mass/Charge 543.3	NA	[30]
2018	IRI	HPLC-FLD	Hospital effluent	Liquid-liquid microextraction, solid-phase extraction	Column: Alltima (Deerfield, USA) C18 (150 × 3.2 mm, 5 µm particle size), Elution–Gradient, Mobile phase-Solvent A–Acetonitrile, Solvent B-(100 mmol Ammonium formate, with 0.02% triethylamine) pH-4.5	NA	Fluorescence	0.8 to 100 µg/l	[14]

Year	Drug	Method	Matrix	Sample preparation	Column/Mobile phase	Retention time	Detection	Linearity range	Ref.
2019	IRI	UPLC-MS-MS	Hospital chemotherapy compounding units	Dilution	Column: Cortecs UPLC T 3, (1.6 µm, 2.1 mm × 100 mm), Elution–Gradient, Mobile phase: freshly prepared 10 mmol ammonium acetate (pH 5.1) (Solvent A) pure ACN as organic modifiers (Solvent B).	10.9 min	Precursor ion 587.3 m/z	1-400 ng/ml	[41]
2013	IRI	LC-MS-MS	Wipe samples	Extraction	Column: Octadecyl silyl column Inertsil® (GL Sciences, Tokyo, Japan) ODS-3; 50 mm × 2.1 mm; particle size 3 µm; guard column (cartridge guard-column® (GL Sciences, Tokyo, Japan) 20 mm × 2.0 mm; particle size, 3 µm)., Mobile phases: 0.1% formic acid–water (Solvent A), ACN (Solvent B)	5.77 min	Mass/Charge 587.7→167.3 (IRI)	5–1000 ng/wipe	[45]
2006	SN-38	HPLC-DAD	Novel liposome-based formulation	Dilution	Column: Agilent Zorbax SB-C18 (4.6 mm × 250 mm, 5 µm), Mobile phase: 25 mmol Sodium dihydrogen phosphate with pH 3.1 and ACN in the ratio 50:50, (%v/v).	4.9 min	265 nm	1–25 µg/ml	[46]
2015	IRI	HPLC-UV	Cancer chemotherapy infusions	Dilution	Column: Synergi® Max-RP, (Phenomenex)C12-(4 µm, 150 mm × 4.6 mm) with guard-column-Max-RP (4 mm × 3 mm)., Elution–GradientMobile phase: Solvent A (6.2 mmol Nonafluoropentanoic acid (NFPA) aqueous solution, with pH 2.5) and Solvent B (MeOH)	16.6 min	254 nm	6–120 µg/ml	[49]
2012	IRI	LC-MS-MS	Wipe samples	Desorption	Column: ZORBAX SB-C18 (RR-2.1 × 100 mm, 3.5 µm), Mobile phase: Ultrapure water (solution A), ACN (solution B) and FA 1% (solution C).	13.66 min	Mass/Charge 587.9→ 587.3	NA	[51]
2007	IRI	RP-HPLC-UV	Injections	Dilution	Column: Reverse phase cyano column (4.6 mm × 25 cm, 5 µm)., Mobile phase: Phosphate buffer with pH 2.5: ACN (75:25) (%v/v).	5.82 min	225 nm	20.0 to 80.0 µg/ml	[52]
2011	IRI	HPLC-UV	Bulk and tablets	Dilution	Column: Inertsil ODS C-18 column-(250 × 4.6 mm, 5 µm), Elution-isocratic mode, Mobile phase-ACN: MeOH: 0.01 M Potassium dihydrogen ortho-phosphate in the ratio of 55:18:27 (%v/v/v)	2.9 min	220 nm	30-90 µg/ml	[2]
2009	IRI HCL	RP-HPLC-UV	Injections	Dilution	Column: Kromasil C18 analytical column-(4.6 mm × 250 mm, 5 µm particle size), Mobile phase-(10 mmol Potassium dihydrogen ortho-phosphate buffer (pH 3.5): ACN: MeOH (55:25:20) (%v/v/v)	7.6±0.18	254 nm	30–70 µg/ml	[53]
2013	IRI	RP-HPLC-UV	Cleaning validation swab samples	Extraction	Column: Waters symmetry shield RP-18-(250 mm × 4.6 mm, 5 µm) column, Elution-Isocratic, Mobile phase-0.02 M Potassium di-hydrogen orthophosphate, with pH 3.5: MeOH: ACN (60:20:20) (%v/v/v).	4.779 min	220 nm	0.024 to 0.143 µg/ml	[55]
2019	IRI (bulk) and dosage forms	RP-HPLC-UV	Bulk and dosage forms	Dilution	Column: Reverse phase Inertsil ODS (250 × 4.6 mm, 5 µm particle size), Mobile phase-0.02 M potassium dihydrogen ortho-phosphate buffer and ACN (40: 60) (%v/v)	2.1 min	222 nm	40-120 µg/ml	[56]
2020	IRI HCl	HPLC-UV	Irinotecan Hydrochloride and Curcumin in Co-delivered Polymeric Nanoparticles	Irinotecan hydrochloride and curcumin co-delivered nanoparticle (ICN) were prepared by combinatorial entrapping them into polyethylene glycol–polylactic acid-co-glycolic acid (PEG–PLGA) polymeric nanoparticles.	Column: C18 column Kinetex-(150 mm × 4.6 mm, 2.6 µm particle size, 100 Å), Pre-column (WATREX 50 mm × 4 mm, ReproSil 100 C18, 5 µm particle size), Mobile phase-ACN and ultrapure water containing sodium dodecyl sulfate (0.08 mol/l), disodium phosphate (Na_2HPO_4, 0.002 mol/l) and acetic acid (4 %v/v) in the ratio 50:50 (%v/v).	3.317 min	256 nm	2.05-1050 µg/ml.	[57]
2012	IRI	UPLC-UV and LC-MS-MS	Injections	Dilution	UPLC: Column: Waters Acquity BEH C8-(100 × 2.1 mm, 1.7 µm), Mobile phase-Solvent A (0.02 mol/l potassium dihydrogen ortho-phosphate, pH with 3.4), Solvent B (a mixture of ACN and MeOH in the ratio of 62:38 %v/v), LC-MS conditions: Column: Waters	1.9-2.4 min (IRI)	220 nm (UPLC) and Mass/Charge 605.5 (LC-MS-	0.063 to 83.2 µg/ml	[58]

Year	Drug	Method	Matrix	Sample preparation	Column/Mobile phase	Retention time	Detection	Linearity range	Ref.
					Symmetry Shield RP 18, -(250 × 4.6 mm, 5 µm) columnMobile phase-Ammonium formate buffer (0.1 mol/l, pH 3.5) (Solvent A) ACN and MeOH in the ratio of 60:40 %v/v, (Solvent B)		MS)		

HPLC-UV

HPLC (High-Performance Liquid Chromatography) is a process where separation is done based on the size, surface charge, and other factors. After separation, UV spectroscopy combined with HPLC allows for the assessment of molecular concentrations. It is straight forward, particularly, accurate, and precise method. To begin, the method was utilised to successfully determine the amount of irinotecan in 100 mg Irnocam pills. In isocratic mode, a mobile phase that contains 55:18:27 (%v/v/v) acetonitrile (ACN): methanol (MeOH): 0.01M Potassium dihydrogen phosphate (KH_2PO_4) was run on an Inertsil ODS C-18, column with a 250 × 4.6 mm, 5 µm internal diameter. At a flow rate of 1.5 ml/min, the effluents were measured at 220 nm. The retention time of irinotecan was 2.9 min. Linearity, accuracy, precision, specificity, detection limit, quantification limit, and resilience were also evaluated. Limit of quantification and limit of detection values was found to be 35 ng/ml and 10 ng/ml, respectively [2, 12].

Biological matrices

A variety of bioanalytical techniques have been developed to estimate the IRI in biological matrices such as blood, plasma, tissue, serum, urine, and so on. By permitting the identification and quantification of substances and their metabolites in sample specimens, bioanalytical techniques aid in the interpretation of the results of bioequivalence, pharmacokinetic, and toxicokinetic studies [15, 16]. Table 1 summarises the many analytical approaches developed for assessing IRI in biological matrices both alone and in combination with other medicines, including hyphenated techniques that allow for faster analysis than previous techniques [2, 8, 9, 11-13, 16-59].

Table 2: Bioanalytical methods for the estimation of IRI in samples

Year	Drug	Method	Matrix	Sample preparation	Column/Mobile phase	Retention time	Detection	Linearity	Ref.
2018	IRI	HPLC-FLD	Dried blood spots	Liquid extraction	Column: Eclipse plus C8 column (150 × 4.6 mm, 5 µm particle size) Mobile phase: 0.1 M phosphate buffer with pH 4.0 and ACN (80:20 %v/v)	8.20 min	Excitation wavelength: 370 nm, Emission wavelength: 420 nm	10 to 3000 ng/ml	[16]
2020	IRI, SN-38	HPLC-FLD	Plasma and tissue homogenates (kidney, spleen, small intestine, liver, colon)	Protein precipitation	Column: Zorbax SB C18 column (150 × 2.1 mm ID, 5 µm particle size) with C18 guard (Phenomenex, USA) column (4 × 2 mm) Elution: Gradient Mobile phase: 20 mmol ammonium acetate buffer with pH 3.5 (Eluent A) ACN (Eluent B)	Between 4.5 to 5.0 min	Excitation wavelength: 368 nm, Emission wavelength: 515 nm	7.5 to 1500 ng/ml (IRI),5 to 1000 ng/ml (SN-38)	[17]
2018	IRI	HPLC-DAD	Plasma	Precipitation of protein sample	Column: Zorbax Extend C18 column (250 mm × 4.6 mm; 5 µm) with security guard column (4.0 × 3.0 mm; 5 µm particle size) Elution: Gradient Mobile phase: Milli Q water (Solvent A) and methanol (Solvent B)	8.97 min	376 nm	0.1 to 1.8 µg/ml	[19]
2019	Irinotecan in a liposomal form (IRI,SN-38 and SN-38G)	Triple quadrapole mass analyser with ESI	Blank plasma, blank liver homogenates, and homogenates mixture of blank tissue (heart, spleen, lung, and kidney)	Solid Phase extraction	Column: UPLC BEH C18 (Waters, USA) column (2.1 mm × 50 mm; 1.7 µm particle size) Elution: Isocratic Mobile phase: ACN: 0.1% formic acid in water (27:73) (%v/v)	0.67 min (CPT-11), 1.63 min (IS) and 1.28 min (SN-38)	m/z 393.2 → 349 (SN-38) m/z 587.4 → 167.1 (IRI) m/z 349.2 → 249.0 (IS)	10 to 10,000 ng/ml (Free IRI), 4.4 to 20,000 ng/ml (Total IRI), 5 to 1000 ng/ml (SN-38), 25 to 1000 ng/ml (Others)	[20]
2016	Irinotecan, SN-38 glucuronide (SN-38G), SN-38	UPLC-MS-MS	Biological matrices-Plasma, urine, faeces, and tissues are all samples that can be taken (liver and kidney)	Liquid-liquid extraction	Column: Acquity UPLC BEH C18 column (2.1 mm × 50 mm, 300 Å, 1.7 µm particle size) Elution: Gradient Mobile phase: 0.1% formic acid (Solvent A) and ACN (Solvent B)	1.8 to 2.0 min (for IRI); 2.1 to 2.2 min (for SN-38); and 1.6 to 1.8 min (for SN-38G)	m/z 587.6-124.04 (for IRI); 393.1-349.06 (for SN-38); and 569.05-393 (for SN-38G)	In plasma samples: 4.88 to 10,000 nM (for IRI), 4.88 to 10,000 nM (for SN-38), 6.25 to 2,000 nM (for SN-38G); In faeces samples: 39 to 5,000 nM (for IRI), 39 to 5,000 nM (for SN-38), 4.88 to 1,250 nM (for SN-38G); In liver and kidney samples: 48.8 to 6,250 nM (for IRI), 48.8 to 6,250 nM (for SN-38), 9.8 to 1,250 nM (for SN-38G).	[21]
2012	IRI, SN-38, and SN-38G	UPLC–MS-MS	Blank human plasma in	Solid-phase extraction	Column: Acquity UPLC BEH RP18 column-(2.1 × 50 mm, 1.7 µm)	0.8 min (IRI), 1.1 min (SN-38G), 1.4 min	Precursor ion m/z: 587.3 (IRI), 393.2 (SN-38), 569.2	5 to 1,000 ng/ml for IRI; 0.5–100 ng/ml for SN-38	[22]

Year	Drug	Method	Matrix	Sample preparation	Column/Mobile phase	Retention time	Detection	Linearity	Ref.
			cryovials		Elution: Gradient Mobile phase: 0.1% formic acid (Solvent A) MeOH (Solvent B).	(Camptothecin), and 1.57 min (SN-38)	(SN-38-G), and 349.1 (Camptothecin). Product ion m/z: 124.2 (IRI), 349.2 (SN-38), 393.2 (SN-38G), and 305.2 (Camptothecin)	and SN-38G	
2010	IRI, SN-38	HPLC-FLD	Plasma	Single-step protein precipitation with ACN	Column: Wakosil II C18 RS column (250 mm × 4.5 mm, 5 µm particle size) Elution: Isocratic Mobile phase: 36 mmol sodium dihydrogen phosphate dehydrate and 4 mmol sodium 1 heptane sulfonate: ACN (72:28) (%v/v)	4.70 min (for IRI), 6.5 min (for SN-38), and 8.00 min for (CPT)	Excitation wavelength: 355 nm, Emission wavelength: 515 nm	5 to 5,000 ng/ml (IRI) and 5 to 240 ng/ml (SN-38)	[23]
2015	IRI	HPLC-UV	Tissue	Tyrode's solution collected after perfusion of intestinal segment and Permeation through everted gut sac	Column: Supelco RP C18 column-(250 mm × 4.6 mm, 5 µm particle size) Elution: Isocratic Mobile phase: 0.045 µM sodium dihydrogen phosphate dehydrate buffer with pH 3.0: ACN (72:28) (%v/v)	8.75±0.15 min	254.9 nm	0.060-10.0 µg/ml	[24]
2016	IRI, SN-38, and SN38-G	HPLC-MS-MS	Blood, Plasma	Protein precipitation	Column: Thermo Scientific Hypersil GOLD column (50 × 2.1 mm, 3 µm particle size) Mobile phase: 0.1% formic acid in water: 0.1% formic acid in ACN (80:20) (%v/v).	4.57 min (for IRI), 4.67 min (for SN-38), and 4.68 min (for Camptothecin)	m/z 587.3→124.1 (for IRI), m/z 393.2→349.3 (for SN-38), and 349.1→305.1 (for Camptothecin)	25–2500 ng/ml (IRI) and 5–500 ng/ml (SN-38)	[25]
2012	IRI, SN-38	UPLC-MS-MS	Plasma and brain	Liquid-liquid extraction	Column: Waters Acqity UPLC BEH C18 column (2.1 mm × 50 mm, 1.7 µm particle size) Elution: Gradient Mobile phase-10 mmol Ammonium acetate buffer (pH 3.5) with 0.1% formic acid (Solvent A) and ACN (Solvent B)	1.8 min (for IRI), 2.1 min (for SN-38), and 2.3 min (for IS)	m/z 587.49→167.11 (for IRI Lactone) m/z 393.37→249.23 (for SN-38), and m/z 349.28→305.30 (for IS)	Both IRI and SN-38: 5–5,000 ng/ml (Plasma) and 1.25–1,250 ng/g (Brain)	[27]
2021	IRI, SN-38, and SN38-G	LC-MS-MS	Plasma and tissue samples	Protein precipitation	Colum: Waters Symmetry C18 (150 × 3.9 mm, 5 µm, particle size) column Elution: Gradient Mobile phase: Mobile phase A-MeOH-water solution (10:90), containing 0.1 % formic acid and 0.1 % ammonium formate (%v/v) Mobile phase B-MeOH solution containing 0.1 % formic acid and 0.1 % ammonium formate	3.01 min (IRI), 3.56 min (SN-38), and 3.03 min (SN-38G).	m/z 605.20 → 543.30 (for IRI), and 411.20 → 347.10 (for SN-38)	For both IRI and SN-38 9–9,000 ng/ml (for Plasma samples) and 10–4,500 ng/ml (for tissue samples)	[28]
2009	IRI, SN-38, SN-38G	LC-MS-MS	Plasma	Protein precipitation	Column: ZORBAX SB (Agilent, USA) C18 column (100 mm × 2.1 mm, 3.5 µm particle size) Mobile phase-water with 0.05% formic acid (Solvent A), ACN (Solvent B)	11.2 min (for IRI), 13.0 min (for SN-38), and 7.3 min (for SN-38G).	m/z 587.3→ 167.1 (for IRI), m/z 393.2→ 349.3 (for SN-38), m/z 569.0→ 393.2 (for SN-38G), and m/z 349.1 → 305.1 (for Camptothecin)	10.0 to 2000.0 ng/ml (for IRI) and 0.5 to 200.0 ng/ml (for SN-38)	[31]
2008	IRI, SN-38	HPLC-UV	Plasma and bile	Single protein precipitation (plasma) and liquid-liquid extraction (bile)	Column: YMC C-18 (ODS-A RP column)-(250 mm × 4.6 mm, 4 µm) stainless steel column. Elution: Gradient Mobile phase: ACN (Solvent A) Milli-Q water, adjusted to pH 3.0 with 20% o-phosphoric acid (Solvent B)	11.4 min (for Topotecan), 13.4 min (for IRI), and 15.5 min (for SN-38)	254 nm and 365 nm (for IRI in plasma and bile, respectively), 380 nm (for SN-38)	For both IRI and SN-38: 25–10,000 ng/ml (Plasma) and 0.5–100 µg/ml (Bile)	[32]
2014	IRI, SN-38	HPLC-MS-MS	Plasma	Protein precipitation	Column: XBridge C18 analytical column (50 × 2.1 mm, 3.5 µ m) Elution: Isocratic Mobile phase: 5 mmol ammonium formate buffer (pH 3.5): ACN (25:75) (%v/v)	1.085 min (for IRI), 1.567 min (for SN-38), and 1.934 min (for Camptothecin)	m/z 587.2→124.1 (for IRI), m/z 393.0→349.0 (for SN-38), and m/z 349.0→305.1 (for Camptothecin)	For both IRI and SN-38: 5 to 1000 ng/ml	[33]
2016	IRI	MALDI-MS, LC-MS-MS	Plasma	Protein precipitation	NA	NA	m/z 587.4 →124.2 (for IRI) m/z	300 to 10,000 ng/ml (MALDI-MS), 10–10,000	[34]

Year	Drug	Method	Matrix	Sample preparation	Column/Mobile phase	Retention time	Detection	Linearity	Ref.
							349.2 → 305.1 (for IS)	ng/ml (LC-MS-MS)	
2008	IRI, SN-38, 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]carbonyloxy-camptothecin (APC)	LC-MS-MS	Human liver microsomal fractions and plasma	Protein precipitation	Column: Alltima C18 column (150 × 2.1 mm, 5 µm) with guard column-5 µm Alltima C18 5 µ (7.5 × 2.1 mm) cartridge. Elution: Gradient Mobile phase: Water (Solvent A) ACN (Solvent B) containing 0.25% formic acid.	4.57 min (for IRI), 4.92 min (for SN-38), 4.53 min (for APC), and 5.09 min (for IS)	m/z 587.20 (for IRI), m/z 392.90 (for SN-38), m/z 619.20 (for APC), and m/z 349.20 (for IS).	Microsomal fractions: 1.56–100 ng/ml (for IRI), 3.13–150 ng/ml (for SN-38), and 0.78–100 ng/ml (for APC) Plasma samples: 1.56–25 ng/ml (for IRI), 3.13–150 ng/ml (for SN-38), and 0.78–25 ng/ml (for APC)	[35]
1999	IRI, SN-38	LC-ES-MS	Serum	Protein precipitation	Column: Symmetry C18, 3.5 µm (150 × 1 mm I.D.) Elution: Gradient Mobile phase-Acetonitrile and 5 mmol Ammonium formate with pH 3.0.	3.9 min (for IRI-Lactone), 3.5 min (for IRI-Carboxylate), 5.1 min (for SN-38–Lactone) 5.5 min (for AN-38–Carboxylate), and 5.4 min (for IS)	m/z 587.3 (for IRI-Lactone), m/z 605.3 (for IRI-Carboxylate), m/z 393.0 (for SN-38-Lactone), m/z 411.2 (for IRI-Carboxylate), and m/z 349.2 (for IS)	0 to 10,000 ng/ml (for IRI), and 0 to 100 ng/ml (for SN-38)	[36]
2014	IRI, SN-38	LC-MS-MS	Plasma, tumours	Tissue homogenization	Column: Reverse phase Waters Xterra® MS C18 column (150 × 2.1 mm) Elution: Isocratic Mobile phase: ACN-MeOH-buffer (5 mmol ammonium formate with 0.1% formic acid) (3:4:3) (%v/v/v)	1.8 min (for IRI), 3.0 (for SN-38), and 3.1 min (for Camptothecin)	m/z 587.6→167.2 (for IRI), 393.6→349.3 (for SN-38), and 349.4→305.2 (for Camptothecin)	50 to 10,000 ng/ml (for IRI), and 10 to 2,500 ng/ml (for SN-38)	[37]
2005	IRI, SN-38, Camptothecin (active lactone and inactive carboxylate forms)	HPLC-TBAHS	Plasma	Protein precipitation	Column: Hypersil ODS Column (200 mm × 4.6 mm, 5 µm) with guard column-Phenomenex C18 Elution: Isocratic Mobile phase: 0.1 M potassium dihydrogen phosphate containing 0.01 M TBAHS (pH 6.4): ACN (75:25) (%v/v).	5.33±0.14 min (for IRI lactone), 11.60±0.20 min (for SN-38 lactone),13.52±0.21 min (for Camptothecin–lactone form), 3.97±0.10 min (for carboxylate form), 7.05±0.15 min (for SN-38 carboxylate form), and 8.12±0.18 min (for camptothecin-carboxaylate form)	Excitation wavelength: 380 nm and Emission wavelength: 540 nm	For all compounds: 0.01 to 10 µM	[38]
2000	IRI, SN-38, Camptothecin	HPLC-FLD	Plasma	De-proteinisation	Column: Nucleosil C18 (Phenomenex) (250 × 4.0 mm, 5 µm particle size) Elution: Isocratic Mobile phase: 0.1 M Potassium dihydrogen phosphate with pH 4.2: ACN (67:33) (%v/v)	0.00 to 5.80 min (for IRI), 5.80 to 7.90 min (for SN-38), and 7.90 to 9.50 (for Camptothecin)	Excitation: 228 nm and Emission: 450 nm (for IRI), 543 nm (for SN-38) and 433 nm (for Camptothecin)	1 to 10,000 ng/ml (for IRI) 0.5 to 400 ng/ml (for SN-38)	[39]
2007	IRI, SN-38, Camptothecin (IS)	HPLC-FLD	Tissue culture media and cancer cells	Solid-phase extraction	Column: Hyperclon (Phenomenex) ODS C18 (200 mm × 4.6 mm) with Phenomenex C18 guard column Elution: Isocratic Mobile phase: ACN: 50 mmol disodium hydrogen phosphate buffer containing 10 mmol sodium 1-heptane-sulfonate, with pH 3.0 with 85% (w/v) ortho-phosphoric acid (27:73), (%v/v)	10.04±1.06 min (for IRI), 6.15±0.06 min (for SN-38), and 7.80±0.07 min (for Camptothecin)	Excitation wavelength: 380 nm and Emission wavelength: 540 nm	5–42,000 ng/ml (IRI) and 1–1500 ng/ml (SN-38) in culture medium and 2–42,000 ng/ml (IRI) and 0.5–1500 ng/ml (SN-38) in cell lysates	[40]
2013	SN-38	Microfluidic chip-based Nano-LC-	Plasma	Protein precipitation	Column: Zorbax 80 SB-C18 (150 mm × 75 µm, 5 µm particle size) Elution: Gradient	5.325 min (for IRI) 5.067 min (for SN-38)	m/z 393.0 → 349.0 (for SN-38), m/z	50 to 10,000 pg/ml	[42]

Year	Drug	Method	Matrix	Sample preparation	Column/Mobile phase	Retention time	Detection	Linearity	Ref.
		MS-MS			Mobile phase: (Solvent A) 0. 1% formic acid in water (Solvent B) 0.1% formic acid in ACN.		349.0 → 305.1 (for IRI)		
2005	IRI, SN-38	LC-MS-MS	Plasma and tissues	Protein precipitation	Method 1: Column: Zorbax (Agilent Technologies, USA) SB-phenyl (2.0 mm × 50 mm, 5 µm) Elution: Isocratic Mobile phase: 20 mmol ammonium acetate with pH 3.5: ACN (65:35) (%v/v) Method 2: Xterra (Waters, USA) C18 (2.0 mm× 50 mm, 5 µm) Elution: Isocratic Mobile phase: 20 mmol ammonium acetate with pH 3.5: ACN (67:33) (%v/v))	In plasma/tissue homogenate: 1.2 to 1.4 min (for SN-38), 1.1 to 1.3 min (for IRI), and 1.4 to 1.6 min (for IS)	m/z 393.4 → 349.3 (for SN-38), m/z 587.6 → 167.2 (for IRI), and m/z 349.3 → 305.3 (for IS)	For both IRI and SN-38: 0.5 to 500 ng/ml	[43]
2019	IRI	HPLC-(MRM-IDA-EPI)	Plasma	Dilution	Column: Xbridge C18 column (150 mm × 4.6 mm, 3.5 µm particle size) Mobile phase: ACN and water, both acidified with 0.1% formic acid	7.67 min	Mass/Charge 587→ 502 (IRI)	0.1–50 ng/ml	[44]
2013	SN-38	LC-MS-MS	Micro dialysates from rat brain	Protein precipitation	Column: Agilent Eclipse Plus RP-18-(2.1 mm × 100 mm, 1.8 µm) Elution: Gradient Mobile phase: ACN: 0.1% methanoic acid	NA	Mass/Charge 393.1→349.1	0.1015-1015 ng/ml	[11]
2003	IR,SN-38,SN-38G,APCand NPC	HPLC-FLD	Plasma and saliva	Protein precipitation	Column: Xterra (Waters, USA) RP18 (250 mm × 4.6 mm, 5 µm) with guard Xterra (Waters, USA) RP18 (20 mm × 3.9 mm, 5 µm) Mobile phase: ACN: sterile water (75:25) (%v/v) (Solvent A) Phosphate buffer, pH 4.0 (Solvent B)	5.54 min (for SN-38G), 13.9 min (for NPC), 16.0 min (for APC), 19.6 min (for IRI) and 22.5 min (for SN-38)	Excitation wavelength: 370 nm, and Emission wavelength: 470 nm for the first 24 min and then at 534 nm for the next 4 min	0.5–1000 µg/l	[47]
2003	SN-38, Camptothecin (IS)	LC-MS-MS	Plasma containing liposome-based SN-38	Protein precipitation	Column: Synergi (Phenomene, USA) Hydro-RP column C18, (50 × 2 mm, 4 µm), Elution-gradient elution Mobile phase: 0.1% acetic acid (Solvent A) and ACN (Solvent B)	1.55 min (for SN-38), and 1.78 min (for IS)	m/z 393.1→349.2 (for SN-38), and m/z 349.1→305.1 (for IS)	0.05–400 ng/ml	[48]
2014	SN-38, Camptothecin (IS)	UPLC–MS-MS	Tumor cells	Extraction	Column: Kinetex (Phenomenex, UK) 1.7 µm PFP, LC Column-(100 mm × 2.1 mm) Elution: Gradient Mobile phase: 0.1% formic acid in water (Solvent A) and 0.1% formic acid in ACN (Solvent B)	1.75 min (for SN-38), and 1.89 min (for IS)	m/z 393.1→349.4 (for SN-38), and m/z 349.1→305.4 (for IS)	0.1–1,000 ng/ml	[9]
2015	IRI, SN-38, camptothecin, (IS), tegafur (TF), 5-fluorouracil (5-FU), and leucovorin (LV)	Capillary electrophoresis with UV and fluorimetric detection	Urine	Solid-supported liquid-liquid extraction	Capillary: Fused-silica (BGB Analytik, Switzerland) capillary of (58.5 cm × 75 µm id × 375 µm od) Solvents for conditioning: 1.0 M NaOH, ultrapure water, and BGE (phosphate buffer, pH 11.34; 20 mmol) Eluted with: Ethyl acetate: methanol ratio (95:5), (%v/v)	I_F: 2.5 to 2.7 min (for IRI), 2.8 to 3.0 min (for IS), and 3.8 to 4.0 min (for SN-38) A_{265}:3.1 to 3.3 min (for IRI), 3.5 to 3.7 min (for IS), and 4.1 to 4.2 min (for TF), 4.5 to 4.7 min (for SN-38), 5.1 to 5.2 min (for 5-FU), and 5.8 5.9 min (for LV)	360 nm (for IRI), 410 nm (for SN-38), 265 nm (for 5-FU), 272 nm (for TF) and 288 nm (for LV) Excitation: 360 nm (IRI) and Emission: 440 nm (IRI). Excitation: 340 and 410 nm (SN-38), and Emission: 548 nm (SN-38)	1 to 30 mg/l (for IRI and LV), 2 to 20 mg/l (for TF and 5-FU) and 0.05 to 3.5 mg/l (for SN-38)	[50]
1997	IRI, SN-38, and Camptothecin (IS)	RP-HPLC-FLD	Plasma	Protein precipitation	Column: Hypersil (LC Service, The Netherlands) ODS Column-(100 × 4.6 mm, 5 µm), With guard column-LiChroCART 4-4 end-capped pre-column (4 × 4 mm, 5 µm) LiChrospher 100 RP-18 material (Merck), Elution: Isocratic Mobile phase: MeOH-0.1 M, ammonium acetate containing 0.01 M tetrabutylammonium sulphate (40:60) (%v/v) for the lactone forms, and (35:65) (%v/v) for the total forms with the pH 5.5	For lactone forms: 4.9 min (for IRI), 8.1 min (for SN-38) and 6.5 min (for IS); and For total forms: 8.3 min (for IRI) and 15.0 min (for SN-38)	Excitation wavelength: 355 nm, and Emission wavelength: 515 nm	For lactone form of IRI and SN-38: 0.5 to 15 ng/ml For total forms of both IRI and SN-38: 2.0 to 200 ng/ml	[13]

Year	Drug	Method	Matrix	Sample preparation	Column/Mobile phase	Retention time	Detection	Linearity	Ref.
					adjusted using hydrochloric acid.				
1998	IRI, SN-38, SN-38G, and 7-ethyl-10[4-N-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecin (APC)	HPLC-FLD	Plasma, urine, and faeces	Protein precipitation	Column: Hypersil (LC Service, Emmen, The Netherlands) ODS (100 × 4.6 mm, 5 µm)with LiChroCart 4-4 end-capped (RP 18) guard column Elution: Isocratic Mobile phase: MeOH: 0.1 M ammonium acetate containing 10 mmol tetrabutylammonium sulphate (30:70), (%v/v), pH adjusted to 5.3 with hydrochloric acid.	6.95±0.21 min (for SN-38G), 11.86±1.27 min (for APC), 16.1±2.55 min (for IRI) and 28.6±1.77 min (for SN-38)	Excitation wavelength: 355 nm Emission wavelength: 515 nm	For plasma: 10 to 400 ng/ml (for IRI and SN-38), and For urine and faeces: 100 to 5,000 ng/ml) (for IRI and SN-38)	[54]
2015	IRI, SN-38, SN-38G, camptothecin (IS) and APC	HPLC-MS-MS	Plasma	Extraction	Column: Gemini (Phenomenex, Torrance, USA) C18 (100 × 2.0 mm, 110 Å, 3 µM) with Security guard cartridge (Gemini-NX C18; 4.0 × 2.0 mm) Mobile phase: 0.1% acetic acid (CH_3COOH)/Bi-distilled water (Solvent A) 0.1% CH_3COOH/ACN (Solvent B)	5.05 min (for IRI), 6.43 min (for SN-38), 7.90 min (for SN-38G), 5.07 min (for APC), and 6.57 min (for IS)	IRI-Mass/Charge 587.4>124.2 SN-38-Mass/Charge 393.3>349.3 SN-38G-Mass/Charge 569.3>393.2 APC-Mass/Charge 619.2>393.3	10 to 10,000 ng/ml (for IRI), 1 to 500 ng/ml (for SN-38 and SN-38G) and 1 to 5,000 ng/ml (for APC)	[59]

DISCUSSION

Irinotecan is a semi-synthetic analogue of camptothecin, a quinolone-based alkaloid found in the native Chinese/Tibetan ornamental tree *Camptotheca acuminata*, also known as Xi Shu in Chinese and Tibetan ("happy tree"). Irinotecan is a prodrug that is metabolised to its active metabolite SN-38 (7-ethyl-10-hydroxycamptothecin) by a carboxylesterase enzyme found mostly in the liver, gut mucosa, and tumour tissue. SN-38 is a powerful inhibitor of the nuclear enzyme topoisomerase I, which is involved in a variety of key nuclear functions, including DNA replication, and is 100 to 1,000 times more hazardous than irinotecan [60] CPT-11 can also be metabolised through cytochrome P-450 (CYP) 3A-mediated oxidation pathways. 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1 piperidino] 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1 piperidino] 7-ethyl-10-carbonyloxycamptothecin (APC) and carbonyloxycamptothecin (APC) (4-amino-1-piperidine). The principal oxidation products catalysed by CYP3A4 are carbonyl oxycamptothecin (NPC), both of which are weak inhibitors of topoisomerase [61].

Irinotecan was discovered and produced in 1983. In the laboratories, it initially demonstrated promising effects against a wide range of cancers [62]. Phase of clinical trials I began my research in Japan in 1986, Europe in 1990, and the United States in 1991. In Japan, irinotecan was first approved in 1994 for the treatment of non-small-cell lung cancer, small-cell lung cancer, and gynaecologic malignancies. In 1995, irinotecan was approved as a second-line treatment for colorectal cancer in France, and a year later in the United States [60].

HPLC with fluorescence or UV detector is the most often used analytical technique [65] for quantifying IRI and its active metabolites in pharmaceutical dosage form, pure form, and plasma [65,66]. TLC, RP-HPLC, HPLC-MS-MS, LC-MS-MS, and UV spectrophotometric approaches have all been reported for quantifying IRI and its metabolites in pure form and in combination with other drugs in bulk and pharmaceutical formulations. In this review article, UV detector wavelengths range from 220 to 400 nm, while fluorescence detector emission wavelengths range from 450 to 550 nm and excitation wavelengths from 355 to 400 nm.

This article reviews several HPLC methods, including HPLC-UV, HPLC-FLD, HPLC-DAD, HPLC-MS-MS, and HPLC-TBAHS; UPLC methods, including UPLC-MS-MS and UPLC-UV; RP-HPLC methods, including RP-HPLC-FLD and RP-HPLC-UV; LC-MS-MS methods, including microfluidic chip-based Nano-LC-MS-MS and LC-MS-MS, MALDI-MS-MS method, capillary electrophoresis with UV and fluorometric detection, and Triple quadrupole mass spectrometer with electrospray ionization methods. Also reviews the simple sample preparation techniques such as solid-phase extraction, liquid-liquid extractions, and protein precipitation, various columns and mobile phase, the retention time of each method, and linearity range of the various methods.

CONCLUSION

Irinotecan is the drug of choice and is frequently used as monotherapy and adjuvant therapy in the treatment of metastatic colorectal cancer. Irinotecan must be estimated in analytical and bioanalytical samples, because it is structurally and pharmacologically distinct from other camptothecin analogues. This study covers a wide range of analytical methods for estimating Irinotecan in analytical and bioanalytical samples as a separate entity, as well as its metabolites as alone and in combination with other anticancer drugs. Over time, a variety of analytical techniques have been developed, including UV visible spectrophotometry, spectrofluorimetric techniques, chromatographic techniques, and hyphenated procedures. LC-MS-MS, HPLC-FLD, HPLC-DAD, HPLC-UV, and RP-HPLC-UV are hyphenated techniques that combine chromatographic and spectral techniques to enable for facile separation and detection of even minute fractions of the drug in both pure and combined form. The recent development in this area includes the development of the sensitive UHPLC-MS-MS method for more accurate and reliable results.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

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