Simultaneous determination of ciprofloxacin hydrochloride and metronidazole in spiked human plasma by ultra performance liquid chromatography-tandem mass spectroscopy

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ABSTRACT

Ciprofloxacin HCl (CIP) and Metronidazole (MET) are antibacterial drugs used in combination for treatment of mixed aerobic/anaerobic infections. An UPLC-MS/MS method was developed for the simultaneous estimation of CIP and MET in spiked human plasma using sildenafil citrate as an internal standard (IS). Protein precipitation was used for analyte extraction. The chromatographic separation was completed within 6 min using a mobile phase of 0.1% formic acid in water and acetonitrile (70: 30, *v/v*), Zorbax C18, 100 x 4.6 mm, 3.5 µm analytical column, at a flow rate of 0.5 mL min^{-1}. Multiple reaction monitoring (MRM) transitions were measured in the positive ion mode. Validation of the method showed standard curves to be linear in the range of 10-4000 ng mL^{-1} for CIP and 30-12000 ng mL^{-1} for MET with mean correlation coefficient exceeding 0.999. In human plasma, CIP and MET were stable for at least 36 days at -70 ± 5 °C, 6 hours at ambient temperature and after three freeze thaw cycles. After extraction from plasma, the samples were stable in auto sampler at 22 °C for 6 hours. The method was simple, specific, sensitive, precise, accurate and suitable for bioequivalence and pharmacokinetic studies.

INTRODUCTION

Ciprofloxacin, [1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazinyl)-quinolone-3-carboxylic acid] (Figure 1a) is a second generation fluoroquinolones member that showed an effective treatment for several systemic infections such as urinary, respiratory, gastrointestinal and cutaneous infections (Wilson *et al.*, 2004). Fluoroquinolones are effective against both Gram-positive and Gram-negative bacteria by inhibiting bacterial DNA gyrase enzyme that leads to inhibition of bacterial DNA synthesis (Wilson *et al.*, 2004). Ciprofloxacin HCl (CIP) is widely used due to its low toxicity as well as its very wide spectrum of antibacterial effect and low ability of causing bacterial resistance. Metronidazole (MET), [2-methyl-5-nitroimidazole-1-ethanol] (Figure 1b), is an antiprotozoal, anti-

amebic and antibacterial drug (Wilson *et al.*, 2004). CIP and MET combination in pharmaceutical dosage forms is used for the treatment of mixed aerobic/anaerobic infections (Werk and Schneider, 1998). Several methods have been reported for the estimation of CIP in biological matrices. Those methods include HPLC operated with UV detection (Khan *et al.*, 2012; Adib *et al.*, 2008; Wu *et al.*, 2008), with fluorescence detection (Ulu, 2011), (Muchohi *et al.*, 2011; Watabe *et al.*, 2010), Liquid chromatography/mass spectroscopy (Imre *et al.*, 2010) and capillary electrophoresis (Bannefeld *et al.*, 1997; Michalska *et al.*, 2004). Similarly, a survey of analytical literature for the determination of MET in biological samples revealed methods based on UV detection using HPLC (Alhalabi *et al.*, 2012), using HPLC-DAD (Ouyang *et al.*, 2010) and LC coupled with tandem mass spectroscopy (Silva *et al.*, 2009). CIP in admixtures with MET and ampicillin has been determined by NMR (Reinscheid, 2006). Also, MET in intravenous admixture with CIP has been

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determined by first-derivative spectrophotometry (Vega and Sola, 2001), second-derivative spectrophotometry (Mahrouse, 2012) and LC (Vega *et al.*, 1999). The pharmacokinetics and serum bactericidal activities of CIP in combination with clindamycin or MET after intravenous administration in volunteers were studied (Deppermann *et al.*, 1989). To the best of our knowledge, no method has been reported for the simultaneous determination of CIP and MET in human plasma using LC/MS/MS. Thus, the aim of the present work was to develop a highly selective and sensitive LC-MS/MS method with good precision and accuracy for the simultaneous estimation of CIP and MET in human plasma. Sildenafil citrate (Figure 1c) was used as an internal standard (IS). A detailed validation of analytical method was performed to yield reliable results. Moreover, the developed method could be successfully applied for further research as to assess the pharmacokinetic evaluation of CIP and MET in combination form.

EXPERIMENTAL

Materials and reagents

Pharmaceutical grade CIP and MET were supplied by MINAPHARM Pharmaceuticals (Cairo, Egypt) and certified to contain 100.70 and 99.44% respectively. Pharmaceutical grade sildenafil citrate (certified to contain 99.80%) was supplied by Pfizer-Egypt. Acetonitrile HPLC grade, formic acid HPLC grade and methanol HPLC grade were all obtained from Sigma-Aldrich, Germany. Human plasma was obtained from VACSERA Blood Bank, Egypt. Deionized and purified water produced in-house from Millipore's Milli-Q System (USA) was used throughout the analysis.

Instrument and conditions

An Agilent ultra- performance liquid chromatograph-mass spectrometer (Germany), equipped with vacuum degasser, gradient quaternary pump VL, auto-sampler ALS, column oven TCC and Agilent (6460 Triple Quadrupole) LC-MS/MS System was used. Separation was carried out on a C18 column (100mm × 4.6mm, 3.5μm) – Zorbax Eclipse Plus, USA. Data acquisition was performed using Agilent MassHunter Workstation software (B.03.01) and data processing was subsequently performed using MassHunter Quantitative analysis software (B.04.00).

Chromatographic elution was performed with a mobile phase consisting of 0.1% formic acid in water and acetonitrile (70: 30, *v/v*), pumped through the column at a flow rate of 0.5 mL min^{-1}. The column temperature was maintained at 30 °C. Under these conditions, the retention times for CIP, MET and IS were around 1.99 min, 2.49 min and 4.89 min, respectively (Figure 2a). The injection volume was 10 μL and the injector needle wash solvent was methanol. The detector was operated in the positive ion mode for the detection of CIP, MET and IS. Multiple Reaction Monitoring (MRM) transitions measured at positive mode at *m/z* 332.205 → 314.0 for CIP (Fig 1d), *m/z* 171.97 → 128.0 for MET (Fig 1 e), and *m/z* 475.1 → 100.2 for IS with a 20 ms dwell time of all drugs. The capillary voltage was set at +4000 V. The sheath gas flow was set at 9.0 L min^{-1} and temperature at 350 °C

with nozzle voltage of 2000 V. Collision energy was set at 20.0, 25.0 and 40.0 V for CIP, MET and IS respectively, while fragmentor voltage was set at 130 V. Quantitation of the analytes in human plasma was based on the peak area ratio of cited drugs to IS.
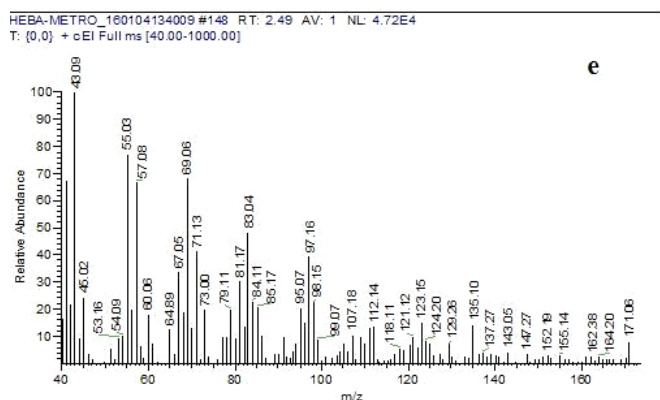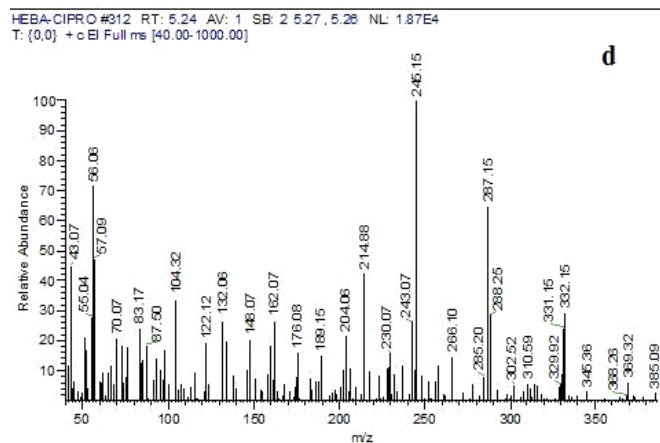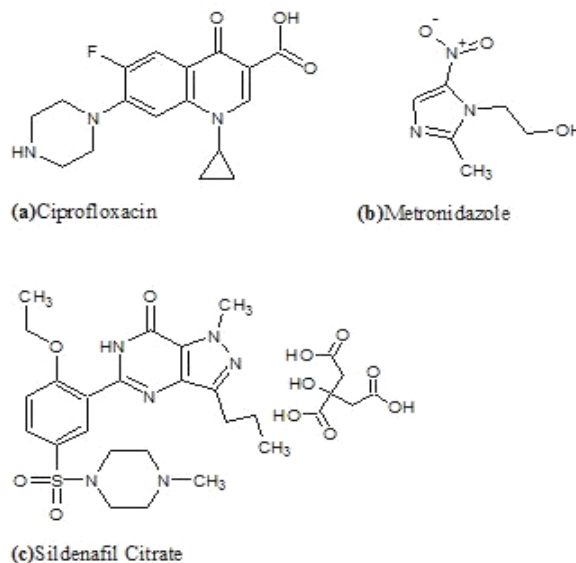
Fig. 1: Chemical structures of ciprofloxacin (a), metronidazole (b) and sildenafil citrate (c). Mass spectra of ciprofloxacin (d) and metronidazole (e).
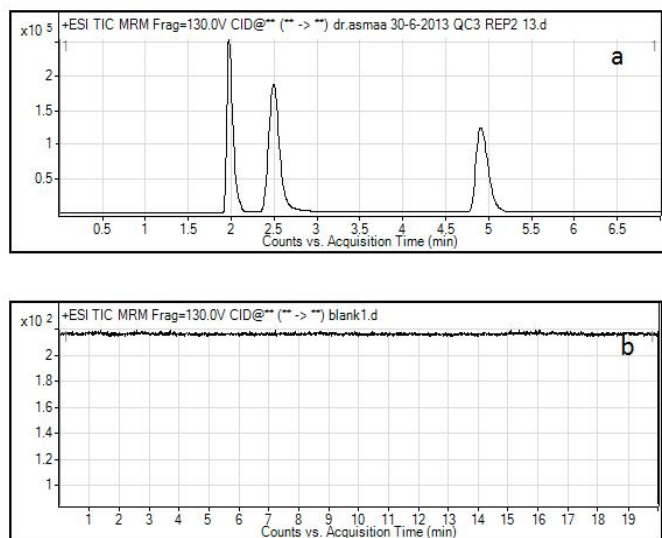
Fig. 2: (a) LC-MS/MS chromatogram of a 10 µL injection of quality control high sample showing elution of ciprofloxacin (CIP) at 1.99min, metronidazole (MET) at 2.49min and IS at 4.89min. (b) LC-MS/MS chromatogram of extracted blank plasma sample.

Stock solution preparation

Stock solutions of 100 µg mL^{-1} of CIP and both (100 µg mL^{-1} and 120 µg mL^{-1}) of MET were separately prepared in methanol-water (50:50, *v/v*). Working solutions of CIP and MET were prepared by serial dilution of the corresponding stock solution with the solvent mixture. Also, IS was prepared in the same solvent mixture at concentration of 20 µg mL^{-1}. All solutions were stored under refrigeration (2-8° C) when not in use.

Calibration samples and quality control samples preparation

Eight non-zero calibration standards ranging from 10 ng mL^{-1} to 4000 ng mL^{-1} for CIP and 30 ng mL^{-1} to 12000 ng mL^{-1} for MET were prepared by adding 50 µL of each known working solution of CIP and MET together, with 50 µL of IS solution to 400 µL of drug free human plasma. The quality control samples (QC) were prepared in the manner similar to the calibration standard at three concentration levels – low, medium and high (30, 1500 and 3000 ng mL^{-1}) for CIP and (90, 5000 and 10000 ng mL^{-1}) for MET, respectively.

All samples were vortexed to ensure complete mixing. During each run, six replicates of QC samples were extracted along with the calibration standards to verify the repeatability of the method.

Sample preparation and extraction procedure

Protein precipitation extraction was used, where 500 µL of acetonitrile was added to 500 µL of each spiked calibration plasma standard or QC sample. The solutions were then vortexed for 1 min and centrifuged at 4000 rpm, for 10 min. The supernatant was transferred to a glass vial for LC-MS/MS analysis.

Validation

Linearity

To establish the linearity, a series of calibration standards of CIP and MET mixture (10, 25, 50, 100, 500, 1000, 2000 and 4000 ng mL^{-1} of CIP mixed with 30, 50, 150, 300, 1500, 3000, 6000 and 12000 ng mL^{-1} of MET , respectively were prepared by adding 50 µL of the respective working solution of both CIP and MET together, and 50 µL of a 20 µg mL^{-1} IS solution to 400 µL of drug free human plasma and analyzed.

Five linearity curves containing eight non-zero concentrations were analyzed. A correlation of more than 0.99 was desirable. The lowest standard within the calibration curve was to be accepted as the lower limit of quantitation (LLOQ) if the analytes response in the standard was five times that of drug free (blank) plasma. In addition, the analyte peak in LLOQ sample should be discrete and reproducible with a precision of ±20.0% and accuracy within 80.0% to 120.0%. The deviation of standards other than LLOQ from the nominal concentration should not exceed ±15.0%. It is desirable that a minimum of six non-zero standards including LLOQ, met the above criteria.

Specificity

Six randomly selected drug free human plasma samples were processed by the similar protein precipitation extraction procedure and analyzed to determine the extent to which endogenous plasma components may interfere with analytes or internal standard.

Recovery (extraction efficiency) from plasma matrix

Recovery of CIP and MET were evaluated by comparing the mean peak responses of five measurements for each extracted quality control (QC) samples of each low, medium and high concentrations to mean peak responses of five unextracted standards of equivalent concentration. Similarly, the recovery of IS was also evaluated at the concentration of 2000 ng mL^{-1}. The recovery of the analytes do not have to be 100.0%, but the extent of recovery of an analyte should be consistent, precise and reproducible (fda.gov, 2004).

Accuracy and precision (intra- and inter-day)

Intra-day accuracy and precision were evaluated by six-replicate analyses of CIP and MET mixture at concentrations of low, medium and high quality control (QC) samples in human plasma. The inter day accuracy and precision were assessed by analysis of low, medium and high quality control samples for CIP and MET on three consecutive days. The precision of the method was determined by calculating the percent coefficient of variation (CV%) for the eighteen determinations obtained for each of the three QC concentrations. For the evaluation of precision, the deviation of each concentration level from the nominal concentration was accepted to be within ±15.0% (except for LLOQ it is ±20%). Similarly, the mean accuracy should not deviate than ± 15.0% of the nominal concentration.

Stability

        Three aliquots of each, low and high QC samples were stored in a freezer at –70 ± 5 °C for 36 days. The samples were then processed and concentrations obtained were compared to nominal concentrations to predict the long-term stability of CIP and MET in human plasma.

        In order to determine the short-term stability of plasma sample three aliquots each of the low and high-unprocessed QC samples were left at ambient temperature (23-30 °C) for 6.0 hours. After 6.0 hours the samples were processed, analyzed and compared with nominal concentrations. Auto-sampler stability was determined by analyzing three aliquots each of low and high QC samples that were processed before storing at 22 °C for 6.0 hours. After completion of 6.0 hours, samples were reanalyzed and concentrations compared with the freshly prepared control samples for cited drugs.

        Effect of freeze and thaw cycles on stability of plasma samples after three freeze and thaw cycles was also determined. Three aliquots each of low and high-unprocessed quality control samples were stored at – 70 ± 5 °C and subjected to three freeze thaw cycles. After the completion of third cycle, the samples were processed, analyzed and results were compared with nominal values.

        All stability samples were considered stable if the deviation from the nominal concentration was within ±15%.

RESULTS AND DISCUSSION

        The aim of the present investigation was to develop a sensitive and fast analytical method for the simultaneous determination and quantification of CIP and MET in human plasma. Based on the C_{max} value of both cited drugs (3000 ng mL^{-1} and 10000 ng mL^{-1}, for CIP and MET respectively (Owens et al., 1997), (Ventura et al., 2008), a suitable linearity range of 10 ng mL^{-1} to 4000 ng mL^{-1} for CIP & 30 ng mL^{-1} to 12000 ng mL^{-1} for MET were selected. The use of conventional C18 column was suitable for optimum separation of CIP and MET when maintained at 30 °C. Tandem mass spectrometric detection allowed the quantification of CIP and MET up to 10 and 30 ng mL^{-1} respectively.

        The aim of the present work was to simultaneously determine both CIP and MET using an UPLC equipped with tandem mass spectrometer to attain higher sensitivity and selectivity for the detection and quantification of cited drugs in human plasma with a fast and cheap extraction process, allowing the method to be used in the pharmacokinetics evaluation of both drugs either alone or in combination.

Method development

        During the optimization cycle, several chromatographic conditions were attempted using C18 column (250 mm×4.6 mm, 5 µm) – Nucleosil and C18 column (100 mm×4.6 mm, 3.5 µm) – Zorbax Eclipse Plus. The use of C18 column with 3.5µm particle

size of packing material under controlled temperature (30 °C) column oven, achieved the desirable peak shape and elution of CIP, MET as well as for IS. Also, the use of a shorter column was of advantage giving a shorter run time, allowing faster batch analysis of human plasma samples.

        Various mobile phase compositions were tried starting with acetronitrile and 10 mM ammonium acetate with 0.1% formic acid in water that showed broadened peak of CIP. By changing the ratios of mobile phase contents, inconvenient broadend peaks were obtained over different flow rates and different formic acid ratios. While using a mobile phase composed of acetonitrile and ammonium acetate in different ratios, tailed broadened peak with inconvenient symmetry were observed. By trying different ratios of mobile phase consisting of acetonitrile and formic acid over different flow rates, it was found that 0.1% formic acid and acetonitrile in the ratio of 70:30 *v/v* using 0.5 mL min^{-1} flow rate gives the good results of sharp well resolved peaks with no overlapping. The use of 0.1% formic acid was to obtain higher detection response through assisting the ionization of cited drug molecules. For sample preparation, extraction methods including liquid-liquid and direct protein precipitation were studied. However liquid-liquid extraction using diethyl ether: dichloro methane mixture, 70:30 *v/v* gave poor recovery results of the analytes. The use of direct protein precipitation extraction method gave the advantage of achieving good sensitivity and fast processing of plasma samples with good recovery results.

Bio-analytical method validation

Linearity and limit of quantitation

        Calibration curves for CIP and MET were constructed by plotting peak area ratio of cited drug to the internal standard versus the concentration of cited drug. The constructed calibration curves were found to be linear and precise over the linearity range of 10 ng mL^{-1} to 4000 ng mL^{-1} for CIP and 30 ng mL^{-1} to 12000 ng mL^{-1} for MET. The lower limit of quantitation were 10 ng mL^{-1} and 30 ng mL^{-1} (lowest standard level) with accuracy of 118.29% and 99.49% for CIP and MET, respectively. Results are presented in Table 1. The regression equation for each cited drug was also computed and the correlation coefficient was found 0.9995 and 0.9996 for CIP and MET, respectively. Back calculations were made from the calibration curves to determine accuracy of each calibration standard (Table 1).

Table 1: Summary of ciprofloxacin and metronidazole calibration standards in human plasma.

Conc. of drug in plasma (ng mL^{-1})		Mean conc. Found (ng mL^{-1})		Accuracy		n[a]
CIP	MET	CIP	MET	CIP	MET	
10	30	11.82	29.84	118.29	99.49	5
25	50	28.36	52.67	113.47	105.34	5
50	150	55.75	150.17	111.5	100.11	5
100	300	110.05	295.35	110.05	98.45	5
500	1500	535.25	1529.32	107.05	101.95	5
1000	3000	929.24	3091.71	92.92	103.05	5

| 2000 | 6000 | 2006.42 | 5822.15 | 100.32 | 97.03 | 5 |
| 4000 | 12000 | 4008.12 | 12058.69 | 100.2 | 100.48 | 5 |

Mean correlation coefficient	CIP	0.9995
	MET	0.9996
Mean Intercept	CIP	0.0034
	MET	0.0081
Mean Slope	CIP	0.0005
	MET	0.0002

a) n, number of determinations.

Specificity

There was no significant interference at the retention times for CIP, MET or IS from six different batches of drug free human plasma used for analysis (Figure 2a, b).

Recovery (extraction efficiency) from plasma matrix

The mean recovery for cited drugs in human plasma ranged between 74.73 to 85.67% and 70.49% to 78.31% for CIP and MET, respectively (Table 2). IS recovery from plasma was found to be 82.21 ± 1.45 (mean ± SD, %).

Accuracy and precision (inter and intra-day)

For CIP coefficient of variation for (Intra-day) was between 0.94% to 7.10% and the accuracy values were found to be between 91.00% to 107.70%. Whereas, for MET coefficient of variation for (Intra-day) was between 0.47% to 5.77% and the accuracy values were found to be between 87.28% and 107.70%. CIP inter-day accuracy ranged from 98.64% to 100.86% with coefficient of variation of 6.04% to 8.04%, while MET inter-day accuracy ranged from 92.10% to 105.93% with coefficient of variation of 1.59% to 6.60%. Results are presented in Tables 3a,b.

Stability

CIP and MET were stable at −70 ± 5 °C for at least 36 days (long term stability) in human plasma. The recovery percents in CIP concentration over the stability-testing period of 36 days in deep freezer at -70 ± 5°C were 97.10% and 90.04% of the concentrations from each of low and high quality control samples, respectively, while the recovery percents in MET concentration

Table 2: Recovery of ciprofloxacin and metronidazole from plasma.

QC sample	Concentration of drug in plasma (ng mL⁻¹)		Mean Recovery (%)		CV (%)ᵃ		nᵇ
	CIP	MET	CIP	MET	CIP	MET	
Low	30	90	74.73	70.49	1.24	0.38	5
Medium	1500	5000	85.67	73.28	1.52	1.24	5
High	3000	10000	79.83	78.31	0.8	1.01	5

a) CV, coefficient of variation; b) n, number of determinations.

Table 3a: Intra-day and inter-day results of ciprofloxacin in human plasma.

	Sample No.	QCL (ng mL⁻¹)			QCM (ng mL⁻¹)			QCH (ng mL⁻¹)		
		Day1	Day 2	Day 3	Day1	Day 2	Day 3	Day1	Day 2	Day 3
	1	29.74	28.66	31.62	1540.71	1638.03	1408.75	2851.14	3130.49	2729.68
	2	30.39	26.95	32.12	1556.52	1687.48	1290.83	2880.58	3256.10	2775.22
	3	29.66	26.24	33.66	1540.98	1458.14	1416.39	2831.60	3258.58	2815.53
	4	28.11	30.91	33.92	1568.51	1616.66	1288.15	2822.03	3083.87	2945.73
	5	29.52	30.69	30.78	1592.09	1602.81	1353.31	2891.14	3078.30	3072.73
	6	29.16	30.68	31.79	1552.21	1643.06	1433.11	2856.33	3257.34	2729.85
Intra-day	Mean.	29.43	29.02	32.32	1558.50	1607.70	1365.09	2855.47	3177.45	2844.79
	SD (±)ᵃ	0.76	2.06	1.22	19.47	78.75	64.40	26.87	89.38	137.33
	Precision as CV%ᵇ	2.58	7.10	3.80	1.24	4.89	4.71	0.94	2.81	4.89
	Accuracy	98.11	96.75	107.73	103.90	107.18	91.00	95.18	105.91	94.82
Inter-day	Mean.	30.26			1510.44			2959.24		
	SD (±)ᵃ	2.03			121.5			179.02		
	Precision as CV%ᵇ	6.70			8.04			6.04		
	Accuracy	100.86			100.69			98.64		

a) S.D., standard deviation; b) CV, coefficient of variation.

Table 3b: Intra-day and inter-day results of metronidazole in human plasma.

	Sample No.	QCL (ng mL⁻¹)			QCM (ng mL⁻¹)			QCH (ng mL⁻¹)		
					Day1	Day 2	Day 3	Day1	Day 2	Day 3
	1	88.35	91.29	79.93	4650.96	4471.90	4321.37	10330.00	10833.95	10624.19
	2	87.72	96.02	87.27	4573.04	4516.51	4176.33	10435.68	10888.26	10618.79
	3	88.53	97.45	78.26	5010.85	4780.89	4259.60	10495.88	10729.93	10664.96
	4	89.92	97.32	78.11	4702.72	4990.01	4228.13	10379.20	10781.37	10647.54
	5	88.68	94.92	87.68	4652.52	4866.68	4295.95	10382.91	10665.79	10520.34
	6	89.94	93.67	85.92	4900.49	4489.35	4381.74	10360.86	10723.35	10601.08
Intra-day	Mean.	88.86	95.11	82.86	4748.43	4685.89	4381.74	10397.43	10770.45	10612.82
	SD (±)ᵃ	0.89	2.36	4.56	266.01	228.08	72.28	59.30	81.04	50.55
	Precision as CV%ᵇ	1.00	2.48	5.51	5.77	4.86	1.64	0.57	0.75	0.47
	Accuracy	98.73	105.68	92.07	94.96	93.71	87.28	103.97	107.70	106.12
inter-day	Mean.	88.94			4605.35			10593.57		
	SD (±)ᵃ	5.87			266.01			168.72		
	Precision as CV%ᵇ	6.60			5.77			1.59		
	Accuracy	98.83			92.10			105.93		

a) S.D., standard deviation; b) CV, coefficient of variation.

Table 4a: Summary of stability data of ciprofloxacin in human plasma.

Stability term	Conc. of CIP (ng mL^{-1})	Mean conc.of CIP found (ng mL^{-1})	S.D.±^{a}	CV (%)^b	Stability (%)	n
Long term (36 days)	30	28.8	0.006	0.02	97.10	3
	3000	2701.24	1.42	0.05	90.04	3
Short term (6 hours)	30	29.01	0.18	0.64	97.81	3
	3000	2660.71	18.86	0.70	97.33	3
Auto-sampler (6hours)	30	29.29	0.18	0.61	98.73	3
	3000	2722.04	7.49	0.27	99.57	3
Freeze and thaw	30	29.48	0.05	1.19	99.37	3
	3000	2730.44	0.54	0.02	99.88	3

a) S.D., standard deviation; b) CV, coefficient of variation; c) n, number of determinations.

Table 4b: Summary of stability data of Metronidazole in human plasma.

Stability term	Conc. of MET (ng mL^{-1})	Mean conc. of MET found (ng mL^{-1})	S.D.±^{a}	CV (%)^b	Stability (%)	n^c
Long term (36 days)	90	85.53	0.68	0.80	96.60	3
	10000	10159.17	50.33	0.49	98.34	3
Short term (6 hours)	90	87.71	0.09	0.1	99.07	3
	10000	10218.25	43.73	0.42	98.91	3
Auto-sampler (6hours)	90	87.58	0.07	0.08	98.91	3
	10000	10400.12	82.25	0.79	100.67	3
Freeze and thaw	90	86.81	0.32	0.37	98.04	3
	10000	10458.29	37.06	0.35	101.24	3

a) S.D., standard deviation; b) CV, coefficient of variation; c) n, number.

over stability-testing for same period under same freezing conditions were 96.60% and 98.34% of the concentrations from each of low and high quality control samples, respectively (Tables 4a,b).

Both CIP and MET were found to be stable over 6.0 hours in human plasma at room temperature (23-30 °C). The stability percents observed for CIP were 97.81% and 97.33% at the concentration of each of low and high quality control samples respectively, while the stability percents observed for MET were 99.07% and 98.91% under same conditions at the concentration of each of low and high quality control samples, respectively (Tables 4a,b). In the auto-sampler at 22 °C, processed samples of CIP and MET were stable for 6.0 hours. Stability percents for samples after 6.0 hours for CIP were 98.73% and 99.57% at low and high quality control levels, respectively, while stability percents for samples at the same conditions for MET were 98.91% and 100.67% at low and high quality control levels, respectively. (Tables 4 a,b).

Frozen plasma samples containing both CIP and MET were found to be stable even after subjecting to three freeze-thaw cycles. The stability percent observed for CIP was 99.37% and 99.88% at low and high quality control levels studied, respectively. While the stability percent for MET after subjecting plasma samples to three freeze-thaw cycles was found to be 98.04% and 101.24% at concentrations of at low and high quality control samples, respectively (Table 4a,b).

CONCLUSIONS

The bio-analytical method presented in this manuscript was specific, sensitive accurate and precise enough to be applied to human clinical pharmacology, bioavailability, and bioequivalence studies requiring pharmacokinetic evaluation. The method required sample preparation by protein precipitation extraction with adequate recovery, followed by isocratic UPLC coupled with tandem mass spectrometric detection (LC-MS/MS). The LC-MS/MS method was rapid enough and capable of simultaneous estimating ciprofloxacin HCl and metronidazole up to 10 and 30 ng mL^{-1}, respectively in human plasma with good accuracy and precision.

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