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Rapid and sensitive determination of selective progesterone modulator ulipristal acetate in human plasma

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ABSTRACT

Ulipristal acetate is a new synthetic selective progesterone receptor modulator developed mainly as emergency contraceptive (EC) and also used for the treatment of uterine fibroids. A cost effective, sensitive, simple and rapid high performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed and validated for the analysis of ulipristal acetate in human plasma. Following liquid-liquid extraction, the analyte (Ulipristal acetate) and internal standard (Levonorgestrel) were chromatographed using mobile phase in an isocratic elution mode on a reverse phase C18 column. The LC-MS/MS operated in multiple reaction monitoring mode for respective $[M+H]^+$ ions, *m/z* 476.2/134.1 for analyte and 313.3/245.1 for internal standard. The assay exhibited linear dynamic range of 1-300 ng/mL. The lower limit of quantification was 1 ng/mL with relative standard deviation of 7.0%. The intra-batch and inter-batch results were precise with coefficient variation of 2.7 to 7.0 (%) and accuracy of 94.2-99.8 (%). The validated method was simple, fast and repeatable for bioequivalence, pharmacokinetic and therapeutic monitoring studies.

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1. Introduction

Despite the availability of highly effective methods of contraception, a great number of pregnancies are unintended. Many women who experience an unintended pregnancy have become pregnant as a result of either lacking of contraceptives or contraceptive failure. Emergency contraception is defined as the use of any drug or device after unprotected intercourse to prevent an unwanted pregnancy. Ulipristal acetate is a derivative of 19-norprogesterone and was developed to have enhanced specificity for progesterone receptor. Pre-clinical studies indicate that ulipristal acetate (UPA) binds to human progesterone, glucocorticoid and androgen receptors at approximately 6, 1.5 and 0.2 times the affinity of the endogenous ligands and shows *in-vivo* anti-glucocorticoid and anti-androgen activity at doses approximately 50-fold greater than those needed for anti-progestin effect [1]. The drug has minimal affinity to androgen receptor and no affinity to human estrogenic or mineralocorticoid receptors [2].

Ulipristal acetate is new drug for EC, approved and marketed in United States, European Union and Latin American countries. It is administered as a one-time 30 mg dose within 120 hours of intercourse, that extending the window of opportunity for EC [3]. It prevents progesterone from binding to its receptor, therefore the gene transcription normally turned on by progesterone was blocked, and the proteins necessary to begin and maintain pregnancy are not synthesized [4]. Ulipristal acetate 5 mg dose is also used for the treatment of uterine fibroids due to its safety and efficacy [5]. Following oral administration ulipristal acetate rapidly absorbed with peak plasma concentration of 176±89 ng/mL, and was extensively metabolized by CYP3A4 in liver and the principal metabolites formed are mono and di demethylated derivatives, of which mono demethylated derivative was pharmacologically active [6]. The measurement of ulipristal acetate itself would suffice for bioequivalence and pharmacokinetic studies that support for regulatory submissions in commercial environment. In literature HPLC method was reported [7] and LC-MS/MS methods used in some clinical trials were reported for determination of ulipristal acetate [5,8], to the best of our knowledge no specific LC-MS/MS method was published till today for analysis of ulipristal acetate in human plasma. In present study, optimized chromatographic conditions were developed for the analysis of ulipristal acetate in plasma samples. The LLOQ achieved is 1 ng/mL and chromatographic runtime was 2 min.

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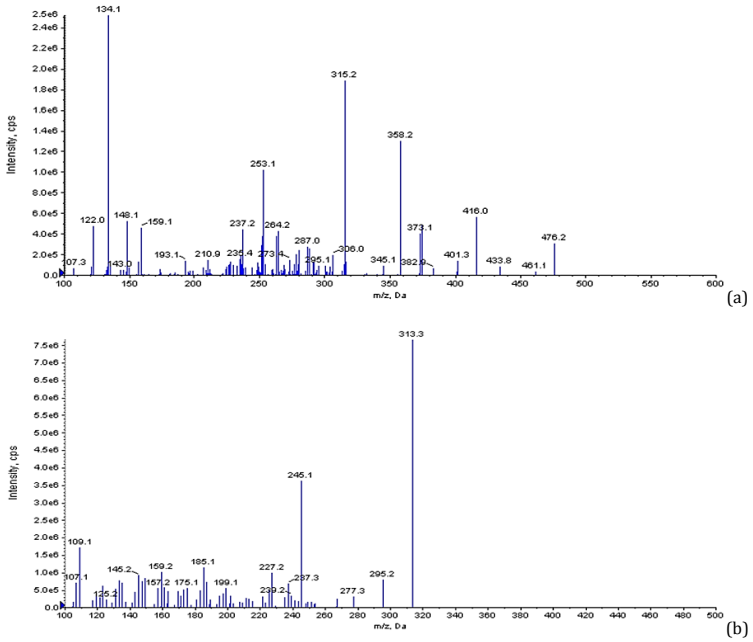

Figure 1. Product ion spectra of (a) ulipristal acetate (*m/z* = 476.2/134.1) and (b) levonorgestrel (*m/z* = 313.3/245.1).

2. Experimental

2.1. Materials and reagents

The ulipristal acetate reference standard and internal standard (ISTD; levonorgestrel) were obtained from Clearsynth Labs. Mumbai, India. Gradient grade LiChro-solv methanol, acetonitrile (ACN), analytical grade ammonium formate, sodium carbonate and formic acid (GR grade) were purchased from Merck specialities (Mumbai, India). Methyl *tert*-butyl ether and *n*-hexane were purchased from Rankem (Mumbai, India). Ultrapure type-1 water from Milli-Q system (Millipore, Bedford, MA, USA) was used for all preparations in the study. Polypropylene RIA vials (ABDOS, New Delhi, India) and volumetric flasks (type A) were obtained from Tarsons products Pvt. Ltd. New Delhi, India. A liquid-liquid extraction VWR multi tube vortexer (Bangalore, India) was used for preparation of samples.

2.2. Mass spectroscopic and chromatographic conditions

The HPLC SIL HTC system (Shimadzu Corporation, Kyoto, Japan) is equipped with an LC-AD VP binary pump, a DGU20A5 degasser, and a SIL-HTC auto sampler with a CTO-10AS VP thermostat column oven maintained at 35 °C temperature. Kinetex 5 µm EVO C18, column (50 mm in length, 4.6 mm internal diameter, and 5 µ particle size) was used as stationary phase. The analyte and internal standard were chromatographed isocratically using mobile phase consisting of a mixture of 10 mM ammonium formate pH = 3.5 (adjusted with formic acid), and acetonitrile (10:90, *v*:*v*). The flow rate was set at 0.6 mL/min. The mobile phase flow was controlled by using split, so that 50% of total flow was injected to the MS system.

Mass spectrometric detection performed using API 4000 triple quadrupole instrument (MDS-SCIEX, Concord, Ontario, Canada) operated in a multiple reaction mode (MRM). Source equipped with turbo ion spray in positive ionization mode, nitrogen gas used as nebulization gas (GS1 35) and heater gas (GS2 35) for desolvation, source temperature was maintained at 500 °C for solvent evaporation. The ion spray needle voltage at 5000 V, entrance potential at 10 V, collision cell exit potential at 4 V, declustering potential at 56 V and collision energy were set at 37 V for both analyte and ISTD. The precursor/product ion transitions in MRM mode were *m/z* 476.2 → *m/z* 134.1 and *m/z* 313.3 → *m/z* 245.1 for analyte and ISTD, respectively. The collision gas and curtain gas were set at 12 and 23 (arbitrary units), respectively. Instrument control, data acquisition and processing was done with Analyst 1.4.2 software (MDS-SCIEX, Concord, Ontario, Canada). The product ion spectra of ulipristal acetate and levonorgestrel are shown in Figure 1a and b. Calibration curves were constructed using a linear fit with $1/x^2$ weighting, which resulted in acceptable accuracy over the linear concentration range.

2.3. Preparation of stock solutions and working dilutions

Stock solution with concentration of approximately 500 µg/mL was prepared in methanol for both analyte and ISTD. Serial spiking dilutions of analyte were prepared in the linear range of 1-300 ng/mL using methanol water mixture (80:20, *v*:*v*) as diluent and 1000 ng/mL of levonorgestrel was also prepared to use as ISTD. Quality control spiking dilutions were also prepared using different stock weighing. All stock solutions and working dilutions are stored in refrigeration at 2-8 °C.

2.4. Preparation of calibration curve and quality control samples

Calibration standards and QC samples were prepared in RIA vial tubes by spiking at 2% (*v*:*v*) of spiking solution into drug-free human plasma containing K_2EDTA as an anticoagulant. Calibration curve samples (CC 1 to CC 8) were prepared across the concentrations range 1-300 ng/mL. QC samples at 5 concentration levels prepared as follows, lower limit of quantification QC (LLOQ QC), 1 ng/mL; low QC (LQC), 3 ng/mL; medium QC (MQC), 126 ng/mL; high QC (HQC), 228 ng/mL and diluted QC (DQC), 600 ng/mL. All calibration and QC plasma samples prepared in RIA vial tubes were stored in a deep freezer at -70 °C until analysis. Before sample prepa-

ration, samples were retrieved from the deep freezer and thawed at room temperature for about 50 min.

2.5. Sample extraction procedure

50 µL of ISTD working dilution (1000 ng/mL) was added to a 300 µL plasma sample in a 15 mL RIA vial tube and vortexed for 10 s. Then add 300 µL of 25 mM sodium carbonate solution and vortexed for 10 seconds. Add 3 mL extraction solvent (*n*-hexane: Methyl *tert*-butyl ether (50:50, *v*:*v*) and vortexed for 10 min at 2000 rpm. After agitation, samples were subjected to freeze for separating the extraction solvent. The separated extraction solvent was evaporated for 20 min under dry nitrogen gas at pressure of 20 psi. After evaporation the samples were reconstituted with 200 µL of mobile phase and 15 µL was injected into LC-MS/MS system for analysis.

3. Results and discussion

3.1. Method development

3.1.1. Selection of internal standard

It is very important to choose an appropriate ISTD to achieve high accuracy and precision in the quantification assay by LC-MS/MS. As generally speaking, isotope labelled internal standards are expensive and escalate the cost of analysis that limits the usage of isotope labelled ISTD. Hence for developing cost effective method, levonorgestrel is selected as internal standard, which was found to have almost similar behaviour (chemical structure and ionisation) and co-eluting properties as analyte. Levonorgestrel also has similar extraction efficiency in optimized conditions and because of its negligible matrix effect renders it suitable as internal standard. Therefore, it was selected as internal standard for all experiments.

3.1.2. LC-MS/MS Conditions

MS/MS parameters were optimized by continuous infusing of analyte and ISTD (500 ng/mL) into electrospray ionisation (ESI) source. It was found that positive-ion mode exhibits greater sensitivity for analyte and internal standard. Tuning and optimization of compound parameters (Declustering potential; DP, Entrance potential; EP, Collision energy; CE, Collision cell exit potential; CXP) was done for selecting ion transitions for both parent and product ions for MRM. Finally source parameters for multiple reactions monitoring (MRM) were optimized by infusing analyte and ISTD along with mobile phase.

The chromatographic conditions were optimized primarily on composition of mobile phase. Analyte and ISTD were injected initially using mobile phase consisting mixture of acetonitrile, 10 mM ammonium formate 70:30 (*v*:*v*) on Zorbax C18 (5 µm, 50×4.6 mm, i.d.). The chromatographic retention was at 4 min and the peak is broad. To achieve good peak shape, the required sensitivity at LLOQ level and to reduce the run time the aqueous buffer was adjusted with formic acid to pH = 3.5 from initial value. Compared to methanol, acetonitrile 90% as organic modifier gives highest sensitivity. Several columns were screened, finally Kinetex EVO C18 (5 µm, 50×4.6 mm, i.d.) was selected as a result of its less retention time with acceptable peak shape.

3.1.3. Optimizing sample preparation

Protein precipitation, LLE has been checked for sample preparation. Protein precipitation is simple but levonorgestrel shows matrix effect over 80%. In consequence, LLE was chosen to prepare samples for benefit of specificity and cleanliness. Methyl tertiary butyl ether, diethyl ether, ethyl acetate-hexane were attempted individually and also as solvent mixtures. Finally *n*-hexane:methyl *tert*-butyl ether in the ratio of 50:50 (*v*:*v*) was selected as extraction solvent because of its high and similar extraction recovery for analyte and ISTD with negligible matrix effect.

3.2. Method validation

Method validation for analysis of ulipristal acetate in human plasma has been carried out in compliance with the US-FDA and ANVISA resolution (Brazil) guidelines [9,10] as per method validation plan. Results were evaluated for precision (CV ≤ 15%; LLOQ and LLOQ QC: CV ≤ 20%) and accuracy (back calculated concentrations within 85-115%; LLOQ and LLOQQC: 80-120% compared to nominal concentrations). All plasma stability experiments were carried out by comparing stability samples against freshly prepared calibration samples. The stability experiments in plasma were considered stable, if the deviation from nominal value (±15%) and precision (CV ≤ 15%) were within the acceptable limits. Prepared stock solutions and working dilutions were considered stable if deviation between stability and comparison (freshly prepared) were within ±10%.

3.2.1. Carryover and cleaning validation

Extracted blank, high standard followed by two extracted blank samples and low standard sample were injected to check the carryover test. Carryover shouldn't be more than 20% and 5% at retention time of analyte and internal standard respectively. All the glassware used in the study was washed with diluent and injected into the system with method conditions to check the presence of potential interferences from glassware materials.

3.2.2. Selectivity

The selectivity of the method was determined to check the potential interferences of endogenous compounds present in biological matrix or co-eluting interferences at the retention time of the analyte and ISTD. Total of 12 human blank plasma samples were processed as per sample extraction procedure as follows: 8 normal, 2 lipemic and 2 heamolytic plasma lots. No interference peaks were observed at the retention time of analyte and ISTD. Identical chromatograms of plasma lots prove assay diversity.

3.2.3. Matrix effect

3.2.3.1. IS normalized factor and direct quantification

The effect of the presence of endogenous matrix components on ulipristal acetate was evaluated in 10 different screened blank plasma lots (6 normal, 2 lipemic and 2 hemolytic) containing K_2EDTA as an anticoagulant. From each plasma lot a total of 6 samples (three replicates each for LQC and HQC) are processed and injected. All back calculated concentrations their average and individual at LQC, HQC were within ±15%.

Each two samples were processed from each blank plasma lot. Samples were reconstituted with respective solutions (post spiking, one LQC and one HQC) prepared in mobile phase to get the equal concentration as extracted samples. Prepare aqueous samples of LQC and HQC having the concentration equal to the post spiked samples. The response ratio of analyte and ISTD in the post spiked samples versus aqueous samples (considering 100% response) was calculated. Mean response ratio (%) and precision (%CV) of the ISTD normalized matrix factor for analyte in K_2EDTA were 0.98 and 6.30 for LQC and 1.01 and 4.80 for HQC, shows that there is negligible ion suppression or enhancement. The results were shown in Table 1.

Table 1. Matrix effect data results, MF-Matrix factor, ISNMF-Internal standard normalised factor.

Blank plasma lots	LQC			HQC		
	MF analyte	MF ISTD	ISNMF	MF analyte	MF ISTD	ISNMF
LOT-1	0.91	0.92	0.99	0.94	0.88	1.07
LOT-2	0.88	0.95	0.93	0.88	0.84	1.05
LOT-3	0.85	0.91	0.93	0.92	0.89	1.03
LOT-4	0.86	0.92	0.93	0.93	0.96	0.97
LOT-5	0.92	0.98	0.94	0.94	0.89	1.06
LOT-6	0.91	0.86	1.06	0.92	0.95	0.97
LOT-7 Hemolytic	0.92	0.85	1.08	0.88	0.94	0.94
LOT-8 Hemolytic	0.86	0.88	0.98	0.86	0.82	1.05
LOT-9 Lipemic	0.89	0.94	0.95	0.92	0.88	1.05
LOT-10 Lipemic	0.91	0.85	1.07	0.92	0.96	0.96
Mean			0.9860	Mean		1.013
SD (Standard deviation)			0.0618	SD		0.0491
%CV (Coefficient of variation)			6.30	%CV		4.80

Table 2. Typical extraction recoveries of analyte and ISTD from solvents and solvent mixtures.

Extraction solvent/Mixture	% Average recovery of analyte	% Recovery of ISTD
Methyl tert-butyl ether (MTBE)	45.5	38.2
Di ethyl ether (DEE)	51.0	65.0
MTBE: DEE (70:30, *v*:*v*)	61.0	74.0
MTBE: Ethyl Acetate (50:50, *v*:*v*)	58.0	63.0
MTBE: *n*-Hexane (50:50, *v*:*v*)	63.0	64.5

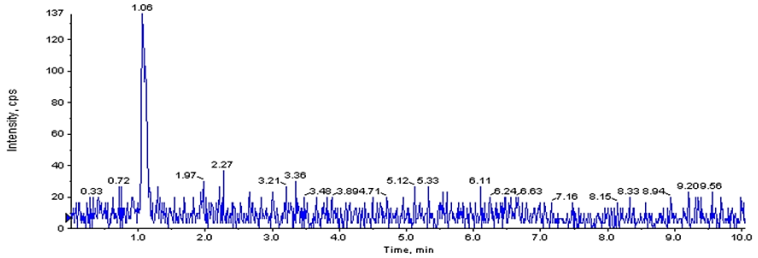

Figure 2. Chromatogram for phospholipid elution and long runs.

3.2.3.2. Direct infusion method

While infusing continues extracted solution from blank matrix, the aqueous solution of analyte at LOQ level was injected to check the effect of matrix ions on intensity and peak shape. There was no suppression or enhancement in the signal was observed.

3.2.3.3. Phospholipid elution and long runs

Phosphoglycerides, source of phospholipids, present in the cell membrane are the major cause of matrix effect in bio analytical methods [11,12]. Extracted blank sample was injected using MRM (*m/z* 184 → *m/z* 184), the most commonly selected MRM to check the typical phospholipids (2-lyso and diradyl phosphocholines) elution. Further, analytical run was extended to 10 min to check long runs and phospholipid elution [12]. The chromatogram represents that there is no elution of phospholipids at the retention time of analyte and ISTD and also in continuous run which represents sample cleanliness. The representative chromatogram for phosphor-lipid elution and long runs was shown in Figure 2.

3.2.4. Lower limit of quantification or sensitivity

The measurable LLOQ is determined at 1 ng/mL concentration. Prepared and processed 6 replicates of spiked LLOQ plasma samples. The Average signal to noise ratio was found to be 80.1. The precision (%RSD or %CV) and accuracy were found to be 7.0 and 94.2%, respectively, indicates method reproducibility at LLOQ level.

3.2.5. Method linearity

Linearity was performed using eight calibration points (except standard blank and standard zero) across the calibration range of 1-300 ng/mL (1, 2, 5, 20, 80, 160, 240 and 300 ng/mL). Standard blank sample with addition of 50 µL diluent and standard zero sample with addition of 50 µL of ISTD are prepared along with calibration curve samples. Both samples were used to check the interferences at analyte and ISTD retention times. The observed interference at the retention time of analyte and ISTD is not more than 20% and 5%, respectively. A linear regression analysis with weighing ($1/x^2$) was used to determine slopes, intercepts, and correlation coefficients. The coefficient of determination (r) was greater than 0.98 for all the curves.

3.2.6. Recovery

The extraction recovery of the analyte and ISTD from human plasma was determined by comparing each six replicates of LQC, MQC and HQC of processed samples with that of respective post spiked samples. The recovery at LQC, MQC and HQC was found to be 61.0, 65.0 and 63.1%, respectively. The overall average recovery of analyte and ISTD was found to be 63 and 64.5%, respectively.

Use of extraction solvents and their combinations (Section 3.1.3) results in variable recoveries for analyte and ISTD, but finally similar recovery for both analyte and ISTD were achieved with proposed extraction solvent which nullifies the matrix effect. The mean observed recoveries of both analyte and ISTD were shown in Table 2.

Table 3. Precision and accuracy of ulipristal acetate in human plasma samples.

Sample name	Conc. added (ng/mL)	Within batch (n=12)			Between batch (n=18)		
		Conc. found (ng/mL) Mean±SD	Precision (%)	Accuracy (%)	Conc. found (ng/mL) Mean±SD	Precision (%)	Accuracy (%)
LLOQQC	1	0.9±0.1	7.0	94.2	1.0±0.1	6.3	95.6
LQC	3	3.0±0.1	3.9	98.6	3.0±0.1	4.0	98.3
MQC	126	127.7±3.5	2.7	98.7	126.4±5.5	4.4	99.7
HQC	228	232.6±8.7	3.7	98.0	232.4±7.3	3.1	98.1
DQC	600	610.9±12.5	2.1	98.2	607.9±14.0	2.3	98.7

The very near recovery of analyte and ISTD in turn indicates similar behaviour of analyte and ISTD. The intensity of analyte and ISTD in presence and absence of biological matrix indicates congener behaviour and levonorgestrel suitability as ISTD. So, the method mimics the characteristics of isotope labelled internal standard. Based on this, we conclude levonorgestrel is suitable internal standard for quantification of ulipristal acetate.

3.2.7. Precision and accuracy

It is evaluated by performing three different batches on two days, each consists of six replicates of five QC concentration levels (LLOQQC, LQC, MQC, HQC, and DQC ng/mL) over calibration range to ensure inter (different days) and intraday (within day) precision and accuracy. The mean back calculated concentrations (accuracy) and their reproducibility (precision) at each concentration level were shown in Table 3.

The intraday (average of 12 replicates) and inter-day (average of 18 replicates) precision (%CV) was less than 7.0% and the accuracy was in the range of 94.2 to 99.7%. The obtained result proves that the method was rugged and reproducible over the proposed analytical range. The representative chromatograms of blank plasma, Lower limit of quantification (LLOQ) and Upper limit of quantification (ULOQ) level for analyte and ISTD were shown in Figure 3 to 5, respectively.

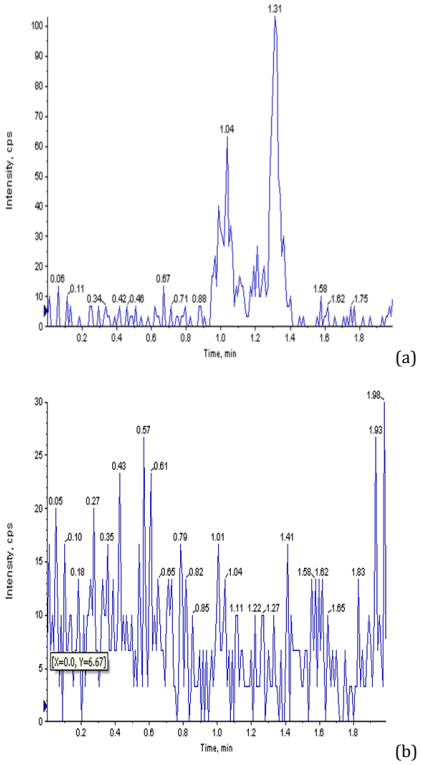

Figure 3. Blank plasma chromatogram of (a) ulipristal acetate and (b) levonorgestrel.

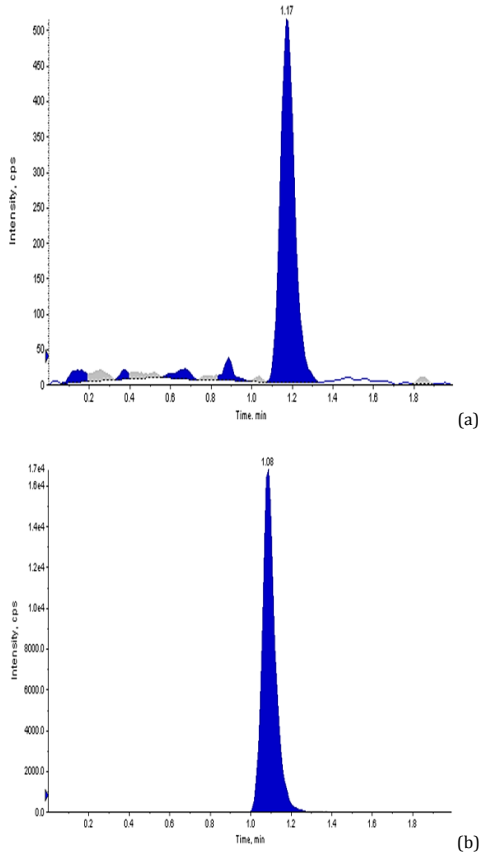

Figure 4. Chromatograms of (a) ulipristal acetate and (b) levonorgestrel at LLOQ level.

3.2.8. Dilution integrity (DQC)

The interference free (screened) plasma lot was spiked to get the concentration approximately double the ULOQ, i.e 600 ng/mL of analyte and the sample was further diluted (1/4) to get DQC sample. The precision and accuracy (n=6) was found to be 2.0 and 98.1%, respectively.

3.2.9. Stability in plasma and processed samples

Stability of the ulipristal acetate in human plasma (Bench top stability, freeze and thaw stability, long term stability in matrix) and in the processed samples (Dry extract stability, In-injector stability and re-injection reproducibility) were assessed as a part of method validation. For all stability experiments, six replicates of LQC and HQC stability samples were processed and evaluated against freshly prepared calibration curve. In case of long term stability experiments fresh stock weighing was done on the day of evaluation. Samples were injected as per method validation working plan. %Stability was indicated based on precision and accuracy results of stability control samples.

Table 4. Stability data of ulipristal acetate under different conditions.

Stability conditions	Conc. added (ng/mL)	Stability (%)	Precision (%)
Ambient temperature 20.67 h	3	99.3	2.3
	228	98.9	3.0
Freeze and thaw cycles	3	99.1	3.9
	228	97.3	3.2
Auto sampler at 5 °C for 29 h	3	97.2	5.1
	228	97.9	2.6
Dry extract at 2-8 °C for 35.98 h	3	99.0	3.3
	228	98.0	3.3
Long term at -70 °C for 42 d	3	98.3	4.6
	228	98.1	3.7

The analyte was considered to be stable in the tested conditions if the precision and accuracy was within ≤15% and ±15% (85-115%), respectively. The stability conditions and results were shown in Table 4.

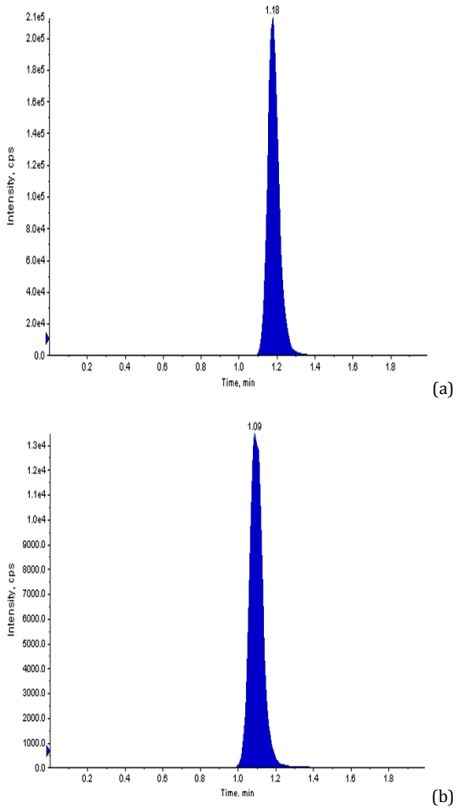

Figure 5. Chromatograms of (a) ulipristal acetate and (b) levonorgestrel at ULOQ level.

3.2.10. Stability in solutions

Stock solution and working solution stability was assessed at room temperature (after 8 to 10 hours) and in refrigerator at 2-8 °C after six days. The % stability was calculated by comparing mean response of six replicates of stability and comparison samples. The stock solutions and working dilutions were stable at proposed conditions.

3.2.11. Concomitant medication

During clinical trial process, investigators may use some over the counter medications to treat unexpected or expected adverse effects like fever, nausea, vomiting etc. Therefore, selectivity and reproducibility of the analytical method was evaluated in presence of concomitant drugs by spiking respective drug dilutions in individual blank plasma samples. Along with this cocktail mixture of concomitant drug dilution was prepared and spiked at LQC level. The concentration of concomitant medication drugs used approximately equal to their C_{max} value. Prepared samples were subjected to extraction along with calibration curve and analysed. The precision and accuracy of LQC samples and observed interference from blank samples at retention time of analyte and ISTD compared to low standard in calibration curve were found to be within acceptable limits. The commonly used drugs as per method validation plan are paracetamol, ibuprofen, aceclofenac, ranitidine and ondansetron.

3.2.12. Whole human blood Stability

Stability of the analyte in whole human blood was evaluated at room temperature. Stability samples at LQC and HQC level in whole human blood were prepared and kept on the working bench. Approximately after 2 hr., comparison samples were also prepared in whole human blood. Plasma was separated from both comparison and stability samples by centrifuging the samples at 3000 rpm at 4 °C for about 20 min. Each six replicates LQC and HQC level from both stability and comparison samples were processed as per sample extraction procedure and analysed. The percentage stability was calculated by comparing the mean area ratios of stability and comparison samples at LQC and HQC levels. The % stability and precision at LQC and HQC were 93.5%, 1.8, and 96.4%, 0.6, respectively.

3.2.13. Extended run (Batch size) precision and accuracy

It is evaluated by processing three control samples on each level (LQC, MQC, HQC) from six different plasma vacutainer sources of K_2 EDTA (Jindal Bio lab, Bio X, JN science tech, BD-Mumbai, SV Lab tech-Hyderabad, Jai bro diagnostics-Delhi), one lot of plasma pool (prepared from screened lots in selectivity) along with each two lots of lipemic and haemolytic plasma. Before spiking, one aliquot of plasma sample was used to screen the plasma lots. The processed control samples were analysed against the calibration curve. The overall accuracy and precision at LQC 95.8, 8.4%, MQC 101.2%, 5.3% and at HQC were 103, 3.4%. The results of this experiment shown that the proposed method was suitable for quantification of ulipristal acetate in clinical samples by eliminating inter subject biological matrix variation.

The total number of control samples processed was more than the total expected sampling time points for human pharmacokinetics/bioequivalence studies of ulipristal acetate. The expected samples were considered based on the pharmacokinetic data of ulipristal acetate [13]. The control samples acceptance in extended precision and accuracy run indicates that method was reproducible and precise for a complete analytical batch run under regulated environment.

3.2.13.1. ISTD trend analysis

The variation in ISTD response will affect the unknown sample concentrations. ISTD variation was calculated for each individual analytical run by calculating the average response area of accepted calibration curve and control samples.

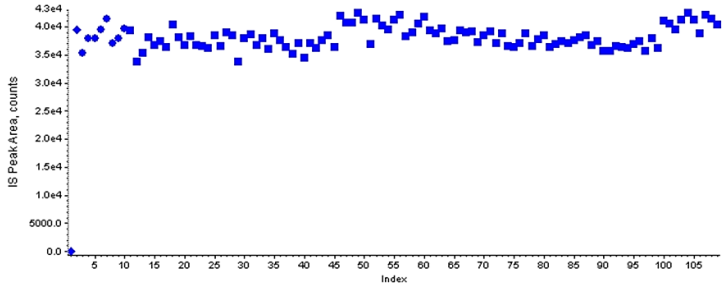

Figure 6. ISTD trend in extended run accuracy.

The unknown sample ISTD response should be within 50-150% of average response. The slow fall or raise in response of ISTD was not considered and addressed in the above format but it will affect the unknown sample concentration by more than 15%.

To know ISTD response trend in overall analytical run, the absolute deviation between the average areas analyte and ISTD were calculated and the deviation is 3.6%. The overall precision for ISTD response is 5.1%. The result of ISTD trend analysis proves method diversity and reproducibility. The ISTD trend in extended run accuracy was shown in Figure 6.

4. Conclusion

For monitoring ulipristal acetate concentration in human plasma, a specific, selective, fast, sensitive and accurate LC-MS/MS method was developed and fully validated in the range of 1-300 ng/mL. The validation result shows that the method is repeatable, reproducible and robust. It can be applied for bioequivalence studies and larger Pharmacokinetic therapeutic monitoring studies in patients and in healthy volunteers.

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References

[1]. Gemzell-Danielsson, K.; Chun-Xia, M. *Int. J. Womens Health*. **2010**, *2*, 53-61.
[2]. Goldstajn, M. S.; Baldani, D. P.; Škrgatić, L.; Radaković, B.; Vrčić, H.; Čanić, T. *Collegium Antropol*. **2014**, *38*, 379-384.
[3]. Richardson, A. R.; Fraidy, N. *Clin. Ther.* **2012**, *1*, 24-36.
[4]. Brache, V.; Cochon, L.; Jesam, C.; Maldonado, R.; Salvatierra, A. M.; Levy, D. P.; Gainer, E.; Croxatto, H. B. *Hum. Reprod.* **2010**, *25*, 2256-2263.
[5]. Pohl, O.; Zobrist, R. H.; Gotteland, J. P. *Reprod. Sci.* **2015**, *22*, 476-483.
[6]. Jadav, S. P.; Parmar, D. M. *J. Pharmacol. Pharmacother*. **2012**, *3(2)*, 109-111.
[7]. Shi, J. X.; Zhu, F. Y.; Zou, Q. G.; Sun, L. L; Wei, P. *Chin. J. New Drugs* **2014**, *7*, 839-852.
[8]. Pohl, O.; Williams, A. R.; Bergeron, C. B.; Gotteland, J. P. *Regul. Toxicol. Pharmacol.* **2013**, *66*, 6-12.
[9]. Guidance for Industry: Bioanalytical method validation, U. S. Department of Health and Human services, Food and Drug administration, May 2001, Rockville, MD, USA.
[10]. Bioanalytical method validation guideline, Brazil, Agenda Nacionaldevigilancia Sanitaria (ANVISA) Resolution-RDC no. 27, May 17, 2012.
[11]. Ye, J. H.; Pao, L. H. *PLoS One* **2014**, *24*, 1-18.
[12]. Ismaiel, O. A.; Halquist, M. S.; Elmamly, M. Y.; Shalaby, A.; Karnes, H. T. *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2007**, *859*, 84-93.
[13]. www.medicines.org.uk/emc/medicine/26068/SPC/#PHARMACOKINETIC_PROPS viewed 05 August 2017.