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ORIGINAL ARTICLE

Quantification of 17-desacetyl norgestimate in human plasma by liquid chromatography–tandem mass spectrometry (LC–MS/MS) and its application to bioequivalence study

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Abstract A rapid and sensitive ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) method was developed and validated for the estimation of 17-desacetyl norgestimate in human plasma using solid-phase extraction technique. 17-desacetyl norgestimate D6 was used as the internal standard. Simple gradient chromatographic conditions and mass spectrometric detection enabled accurate and precise measurement of 17-desacetyl norgestimate at sub-picogram levels. The proposed method was validated for a linear range of 20–5000 pg/mL with a correlation coefficient ≥ 0.9988. The intra-run and inter-run precision and accuracy were within 10%. The overall recoveries for 17-desacetyl norgestimate and 17-desacetyl norgestimate D6 were 96.30% and 93.90%, respectively. The total run time was 4.5 min. The developed method was applied for the determination of the pharmacokinetic parameters of 17-desacetyl norgestimate following a single oral administration of a norgestimate and ethinyl estradiol 0.250 mg/0.035 mg tablets in 35 healthy female volunteers.

1. Introduction

17-desacetyl norgestimate is a metabolite of norgestimate (NGM). Norgestimate [(17R)-17-(acetyloxy)-13-ethyl-18, 19-dinor-17-pregn-4-en-20-yn-3-one oxime] is a synthetic progestational

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hormonal contraceptive, which regulates ovulation and menstruation [1–6]. Amongst the most commonly used oral contraceptives are the combination types that contain both a synthetic estrogen (ethinyl estradiol) and a synthetic progestin (norgestimate). Norgestimate and ethinyl estradiol are well absorbed following oral administration. These oral contraceptives hormones are metabolized by the hepatic enzyme cytochrome P450 3A4 (CYP3A4) [7–9]. NGM is metabolized to two active metabolites: its active metabolites 17-desacetyl norgestimate [DesAc-NGM] and norgestrel [NG]. The rapidity and completeness of these reactions mean that circulating concentrations of NGM are extremely low and probably contribute little to activity. On the average, peak serum concentrations of norgestimate are observed within two hours (0.5–2 h for norgestimate) after administration followed by a rapid decline due to distribution and elimination. As the norgestimate serum concentrations following single or multiple dosing are generally below assay detection within 5 h, a major norgestimate serum metabolite, 17-desacetyl norgestimate (which exhibits a serum half-life ranging from 12 and 30 h), appears rapidly in serum with concentrations greatly exceeding that of norgestimate. The 17-deacetylated metabolite (pka 12.22) is pharmacologically active and the pharmacologic profile is similar to that of norgestimate [10–14].

Reported literature has mentioned, to assess the effect of anticonvulsants [8] and rosuavastatin [10], the pharmacokinetics of 17-desacetyl norgestimate and plasma was analyzed for 17-desacetyl norgestimate with validated LC–MS/MS method, but analytical method details are not available for monitoring plasma levels. Only pharmacodynamic properties are available in most of the literature. So for monitoring of 17-desacetyl norgestimate pharmacokinetics in clinical trials and bioequivalence/bioavailabilty study, it was necessary to develop a simple, specific, rapid, selective and sensitive analytical method for the quantification of 17-desacetyl norgestimate in human plasma.

The only LC–MS/MS method reported by Wong et al. [15] was used for determination of 17-desacetyl norgestimate requiring 1 mL serum with double extraction with a large amount of solvent consumption, double centrifugation and evaporation steps. The total mentioned run time was 10.5 min with an injection volume of 70 µL with less sensitivity ranging from 0.1 to 5 ng/mL. As the described method was cumbersome and with the given time and speed challenges for a typical BA/BE turnaround timelines, it was felt necessary to develop a simple, rapid and sensitive analytical method for the quantification of 17-desacetyl norgestimate.

This paper describes the development and validation of an LC–MS/MS method for the quantification of 17-desacetyl norgestimate in human plasma having reduced analytical run time with a lower limit of quantification (LOQ) 20.221 pg/mL.

2. Experimental

2.1. Chemicals and reagents

The analytical standards of 17-desacetyl norgestimate and 17-desacetyl norgestimate D6 were obtained from Clearsynth (Mumbai, India). High-purity water was prepared in-house using a Milli-Q water purification system obtained from Millipore (Bangalore, India). Gradient grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). LC/MS-grade formic acid was purchased from Fisher scientific (Geel, Belgium). Drug-free (blank) buffered human plasma was obtained from Drug Monitoring Research Institute (Mumbai, India) and was stored at −20 °C prior to use.

2.2. Calibration curves

Stock solutions of 17-desacetyl norgestimate and internal standard, 17-desacetyl norgestimate D6, were prepared in methanol at a concentration of 250 µg/mL and 100 µg/mL, respectively. Secondary and working standard solutions were prepared from stock solutions by dilution with methanol/water (50/50, v/v) using a serial dilution method. These diluted working standard solutions were used to prepare the calibration curve and quality control (QC) samples in human plasma.

A ten-point standard calibration curve for 17-desacetyl norgestimate was prepared by spiking 2% of 17-desacetyl norgestimate working standard solution in the blank plasma. The calibration curve ranged from 20.221 to 5002.914 pg/mL. Quality control samples were prepared at four concentration levels—20.221 pg/mL for Lower Limit of Quantification Quality Control (LLOQQC), 60.085 pg/mL for Low Quality Control (LQC), 2036.767 pg/mL for Medium Quality Control (MQC) and 3916.860 pg/mL for High Quality Control (HQC) samples for 17-desacetyl norgestimate from the stock solutions.

2.3. Sample preparation

A 0.5 mL aliquot of 17-desacetyl norgestimate spiked on human plasma sample was mixed with 50 µL of internal standard working solution equivalent to 60 ng/mL of 17-desacetyl norgestimate D6. Then, 0.5 mL of 1% formic acid was added and vortexed to mix. The sample mixture was loaded into an Oasis HLB (1 cm^3/30 mg), extraction cartridge that was preconditioned with 1.0 mL methanol followed by 1.0 mL water. The extraction cartridge was washed with 1.0 mL water followed by 1.0 mL of 20% acetonitrile in water. Both 17-desacetyl norgestimate and 17-desacetyl norgestimate D6 were eluted with 1 mL of methanol; 10 µL of the sample was injected into the LC–MS/MS system.

2.4. Instrumentation

Chromatographic separation was carried out on Waters UPLC with Zorbax Eclipse XDB-C_{18} (150 mm × 4.6 mm, 5 µm) purchased from Agilent, the United States. A degassed mobile phase consisting of acetonitrile (A) and 0.1% formic acid in water (B) was delivered with a flow rate of 0.75 mL/min using a time and solvent composition gradient. Each analytical run was started at 50% A up to 0.3 min followed by a liner gradient to 90% A over 0.5 min, held at 90% A for 2.7 min, shifted to linear to 50% A over 0.5 min, and then held constant until the end of the run for column equilibration to take on the next analytical run. The total run time for each sample analysis was 4.5 min. The column oven temperature was kept at 40 °C. Mass spectra were obtained using an Xevo TQ-S mass spectrometer, a triple-stage quadrapole-mass-analyzer with photomultiplier detector equipped with electrospray ionization (ESI) source (Waters Ltd., UK) running on positive ion mode. The mass spectrometer was operated in the multiple reaction-monitoring (MRM) scan mode. The data acquisition was ascertained by MassLynx 4.1 software.

2.5. Validation

The method was validated for specificity, selectivity, sensitivity, linearity, precision, accuracy, recovery, stability and matrix effect meeting the global regulatory requirements [16,17]. Specificity was performed by analyzing human female blank plasma samples from different sources (or donors) to test for interference at the retention time of 17-desacetyl norgestimate and internal standard, 17-desacetyl norgestimate D6. Selectivity was performed by spiking concomitant drugs like ranitidine, paracetamol, ibuprofen and aspirin. The plasma samples were then processed and analyzed to investigate possible interference. Sensitivity was determined by analyzing six replicates of blank human plasma spiked with the analyte at the lowest level of the calibration curve. The intra-run and inter-run accuracy were determined by replicate ($n=6$) analysis of three quality control samples and at LOQ that were extracted from the sample batch. The intra-run (within batch) precision and accuracy were evaluated by analysis of six replicates at four concentrations in a same analytical run. The inter-run precision and accuracy of the calibration standards were assessed using five calibration curves used for assay validation. The inter-run (between-batch) precision and accuracy were evaluated after repeated analysis in five different analytical runs in different days and on different instrument.

Accuracy was defined as the percent of relative error (RE) and was calculated using the formula RE $(\%)=(E-T)\times(100/T)$, where E is the experimentally determined concentration and T is the theoretical concentration. Assay precision was calculated by using the formula RSD $(\%)=(SD/M)\times 100$, where RSD is the relative standard deviation, M is the mean of experimentally determined concentrations, and SD is the standard deviation of M.

The extraction efficiencies of 17-desacetyl norgestimate and 17-desacetyl norgestimate D6 were determined by comparing the peak area of extracted analytes to the peak area of non-extracted standards (analyte spiked post extraction in blank plasma).

The processed sample stability was evaluated by comparing the freshly extracted plasma samples which were injected immediately (time 0), with the samples that were re-injected after kept in the autosampler at 10 °C for a specific duration of time. The stability of spiked human plasma stored at room temperature (bench-top stability) was evaluated by comparing the mean of back-calculated concentrations of the samples kept on bench with freshly prepared extracted samples. The freeze–thaw stability was conducted by comparing the stability samples that had been frozen and thawed five times stored at −30 and −75 °C, with freshly spiked quality control samples. The long-term stability of spiked human plasma was evaluated by analyzing low-, medium- and high-quality control samples that were stored at −30 and −75 °C for a long duration together with freshly spiked calibration standard and quality control samples. Stability was determined by calculating the change and was calculated using the formula $Change\ (\%)=(S-C)\times 100/C$, where S is the mean stability sample concentration and C is the mean freshly prepared or comparison sample concentration. Analytes were considered stable if the Change was within ±15% of the freshly prepared or comparison sample.

Matrix effect was evaluated with eight different lots of plasma containing K_3EDTA as anticoagulant including one hemolysed lot and one lipemic plasma lot. Three post-spiked samples at each of LQC, MQC and HQC levels were prepared from different lots of plasma (in total 24 samples). Aqueous (unextracted derivatized) spiked samples for 17-desacetyl norgestimate and 17-desacetyl norgestimate D6 at LQC, MQC and HQC levels were prepared in elution solution considering zero matrix effect. The post-spiked extracted LQC, MQC and HQC samples along with six replicate injections of aqueous un-extracted samples at LQC, MQC and HQC levels were analyzed. The matrix effect was evaluated by calculating the matrix factor for area response of 17-desacetyl norgestimate and 17-desacetyl norgestimate D6 and IS normalized matrix factor for mean area ratio of 17-desacetyl norgestimate and 17-desacetyl norgestimate D6.

This was performed with the aim to observe the matrix effect of these different lots of plasma on the %RSD of mean matrix factor for analyte area, IS area and IS normalized area ratio. It was considered there was no matrix effect if the %RSD for mean matrix factor was less than 15% at each level for analyte area, IS area and IS normalized area ratio.

3. Results and discussion

3.1. Tuning and chromatography optimization

To develop a rapid, selective, sensitive and simple assay method for the extraction and quantification of 17-desacetyl norgestimate during method development, different options were evaluated to optimize extraction, chromatography separation and MS detection parameters.

The extraction was first tried by precipitating the proteins in spiked plasma like acetonitrile, methanol and acidic methanol. Further, trials were taken by liquid-liquid extraction with traditional protocol using ethyl acetate, diethyl ether, t-butyl methyl ether and a combination of ethyl acetate and dichloromethane. The recoveries obtained were low but consistent; however, the response for LOQ was not quantifiable and there was significant interference observed from the plasma matrix. After the use of different SPE cartridges, a thorough sample cleanup could be achieved by using Hydrophillic–Lipophillic balanced (HLB) reverse-phase cartridges. The extraction methodology had ensured a better recovery with no matrix effect and no interference was observed for both 17-desacetyl norgestimate and 17-desacetyl norgestimate D6 analytes.

Further optimization in chromatography conditions resulted in improvement in signal, base-line noise and reduced run time. It was also observed that reduction in buffer pH resulted in improved response and peak symmetry. Use of Zorbax Eclipse XDB-C_{18} (150 mm × 4.6 mm, 5 μm) column enabled use of high flow rate, which resulted in run time as low as 4.5 min with better peak symmetry and signal of analytes. The optimized detection and sample extraction chromatography were enabled to reduce processing and analysis time without compromising the sensitivity.

Electro spray ionization (ESI) and atmospheric pressure chemical ionization (APCI) were evaluated to obtain better response of 17-desacetyl norgestimate which accepted the proton in an acidic mobile phase and produced a protonated precursor ion ($[M+H]^+$) at m/z 328.4. It was found that the best signal was achieved with ESI positive ion mode. Product ion m/z 328.4 of 17-desacetyl norgestimate was monitored, which gave better sensitivity and selectivity. The final MRM parameters of 17-desacetyl norgestimate and its internal standard were set at m/z 328.4/124.1 and 334.3/91.1, respectively. The selected fragment ions of each compound, as product ion to be monitored, are indicated in Fig. 1.

3.2. Specificity

Utilization of predominant product ions for each compound enhanced the mass spectrometric specificity. The mass transition

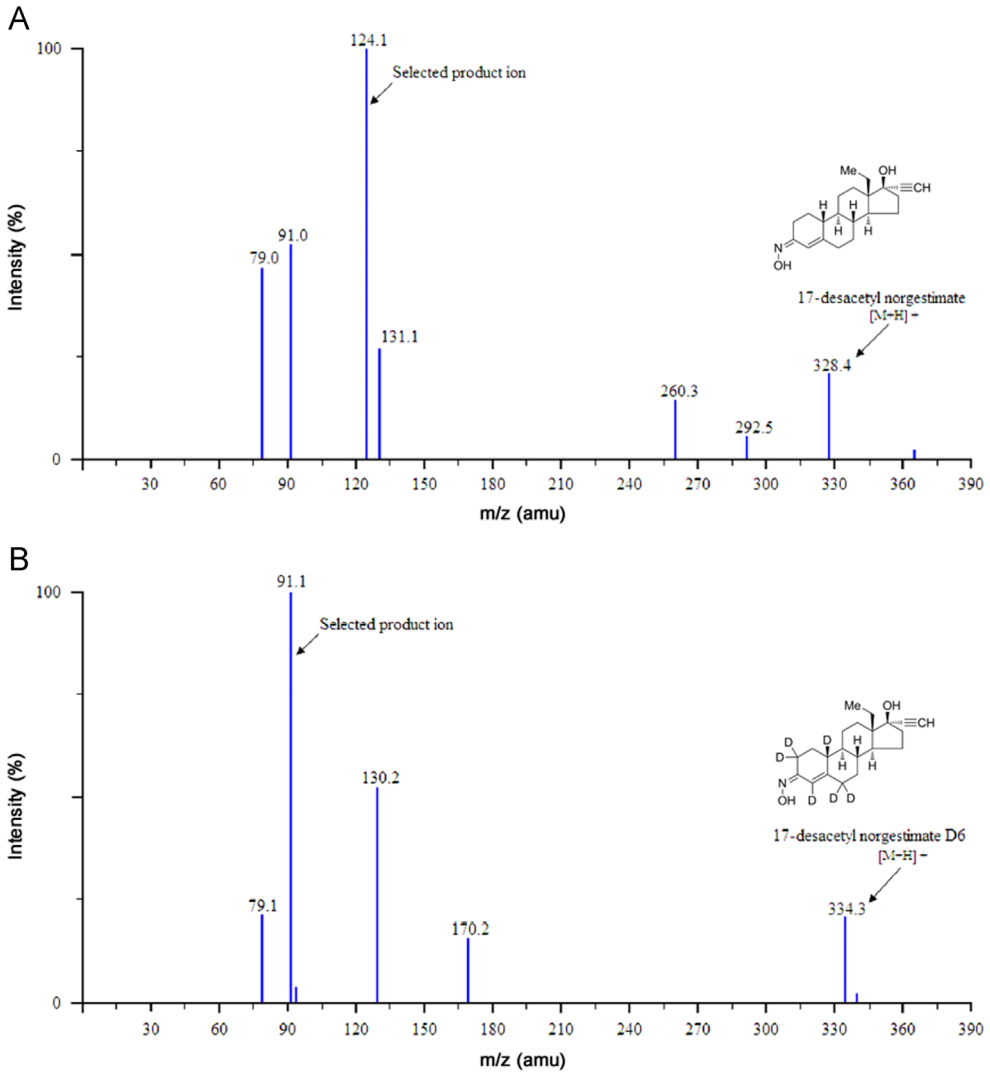

Fig. 1 ESI product ion mass spectra of the precursor ions of (A) 17-desacetyl norgestimate and (B) 17-desacetyl norgestimate D6.

ion-pair was selected as 328.4→124.1 for 17-desacetyl norgestimate and 334.3→91.1 for 17-desacetyl norgestimate D6. The product ions selected were specific for derivatized 17-desacetyl norgestimate and 17-desacetyl norgestimate D6, respectively.

Chromatographic specificity of the method was demonstrated by the absence of endogenous interfering peaks at retention times of 17-desacetyl norgestimate and its internal standard in 10 different lots of extracted blank plasma including one haemolysed and one lipemic plasma. Representative chromatograms of extracted blank plasma and extracted plasma samples containing 20.221 pg/mL 17-desacetyl norgestimate (low standard) are presented in Fig. 2.

3.3. *Linearity*

The peak area ratios (area of 17-desacetyl norgestimate/area of 17-desacetyl norgestimate D6) of calibration standards were proportional to the concentration of analytes in each assay over the nominal concentration range of 20.221–5002.914 pg/mL for 17-desacetyl norgestimate. The calibration curves were found to be linear and were well described by least squares lines. A weighting factor of 1/concentration^2 was chosen to achieve homogeneity of variance. The correlation coefficients were ≥ 0.9988 ($n=5$) for 17-desacetyl norgestimate. The mean ($\pm$SD) slopes of the calibration curves ($n=5$) for 17-desacetyl norgestimate were 0.000933037 ($\pm$0.0000460). The mean accuracy and precision for back-calculated concentrations of each standard calculated from calibration curve are tabulated in Table 1.

3.4. *Sensitivity (lower limit of quantification)*

The LOQ is defined as the lowest concentration of the calibration standard yielding accuracy $\pm$20% and precision of $\leq$20%. The LOQ for 17-desacetyl norgestimate was 20.221 pg/mL. These data are tabulated in Table 2. The intra-run precision at the LOQ plasma samples containing 17-desacetyl norgestimate was 1.62%. The intra-run accuracy (%RE) at the LOQ plasma samples containing 17-desacetyl norgestimate was 3.76%.

3.5. *Precision and accuracy*

The intra-run precision was $\leq$5.02% and intra-run accuracy was $\leq$3.76 for 17-desacetyl norgestimate (Table 2). The inter-run precision and accuracy were determined by pooling all individual assay results of replicate ($n=6$) QC samples over the five separate

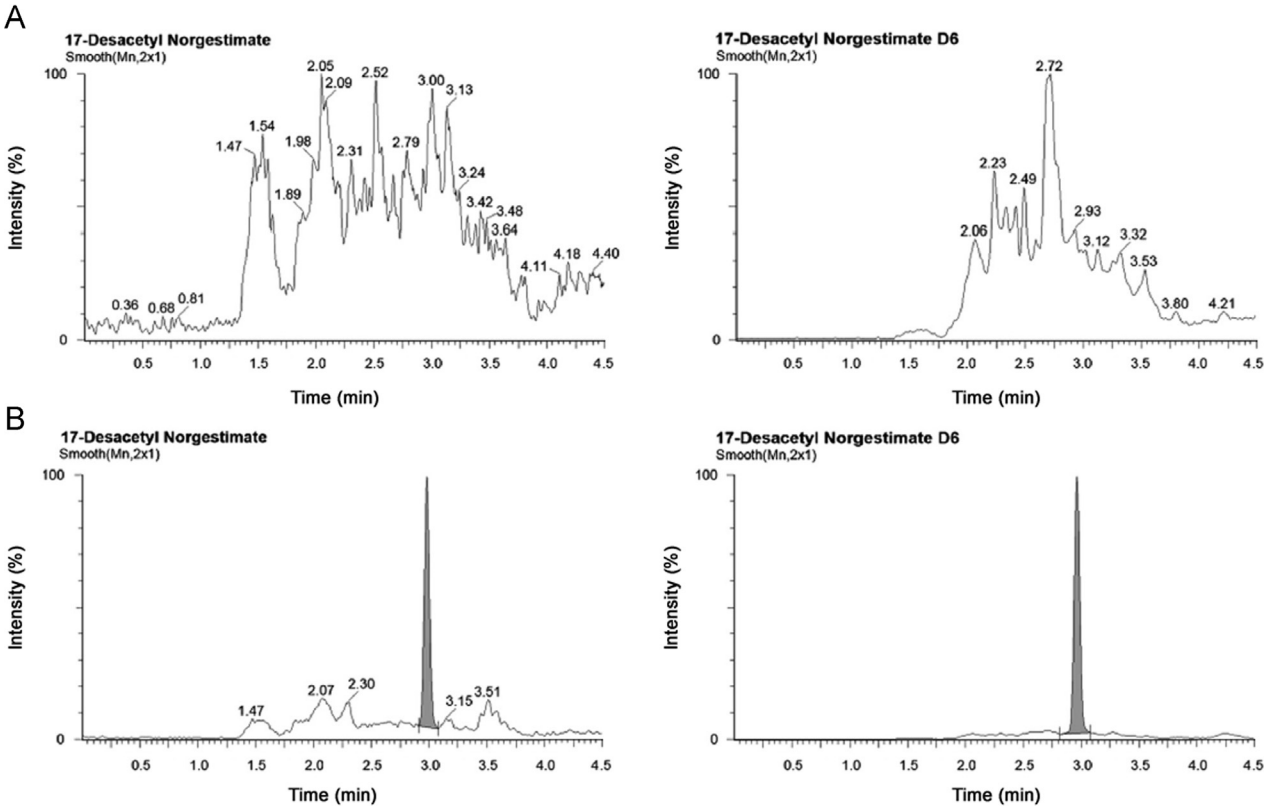

Fig. 2 Representative chromatograms of (A) extracted blank plasma sample and (B) extracted plasma LLOQ sample.

Table 1 Calibration curve back-calculated concentrations of 17-desacetyl norgestimate ($n=5$).

Standard conc. (pg/mL)	Mean calculated conc. ±SD (pg/mL)	RSD (%)	RE (%)	Slope	Intercept	Correlation coefficient (r)
20.221	21.034±0.516	2.45	4.02	0.000933037	0.00026	0.9988
40.442	37.489±1.764	4.71	−7.30			
128.388	123.539±6.529	5.28	−3.78			
256.775	261.107±10.713	4.10	1.69			
513.55	518.500±4.367	0.84	0.96			
1027.101	1051.524±22.360	2.13	2.38			
2054.202	2071.613±49.958	2.41	0.85			
3020.885	2935.282±148.255	5.05	−2.83			
4027.846	4031.551±71.350	1.77	0.09			
5002.914	5199.119±356.318	6.85	3.92			

Table 2 Intra-run (within-batch) and inter-run (between-batch) precision and accuracy of 17-desacetyl norgestimate in human plasma ($n=6$).

Spiked conc. (pg/mL)	Intra-run precision and accuracy			Inter-run precision and accuracy		
	Mean calculated conc. ±SD (pg/mL)	RSD (%)	RE (%)	Mean calculated conc. ±SD (pg/mL)	RSD (%)	RE (%)
20.221	20.981±0.339	1.62	3.76	20.471±1.245	6.08	1.24
60.085	57.647±2.896	5.02	−4.06	58.593±3.660	6.25	−2.48
2036.767	2064.948±21.480	1.04	1.38	2048.910±58.157	2.84	0.60
3916.860	3822.582±168.806	4.42	−2.41	3886.195±158.228	4.07	−0.78

batch runs. The inter-run precision was ≤6.25. The inter-run accuracy was ≤1.24 for 17-desacetyl norgestimate (Table 2).

3.6. Recovery

Six replicates at low-, medium- and high-quality control concentrations for 17-desacetyl norgestimate were prepared for recovery determination. The mean recovery for 17-desacetyl norgestimate was 96.30% with precision of 5.47%. The mean recovery for 17-desacetyl norgestimate D6 was 93.90%.

3.7. Stability

The results of the stability studies are enumerated in Table 3. The bench-top stability, process stability, freeze–thaw stability and long-term stability in matrix of 17-desacetyl norgestimate in plasma were investigated by analyzing quality control samples in replicates ($n=6$) at LQC, MQC and HQC levels. Process stability results indicated that the difference in back-calculated concentration from time 0 to 60 h was ≤3.44%, which allowed one to

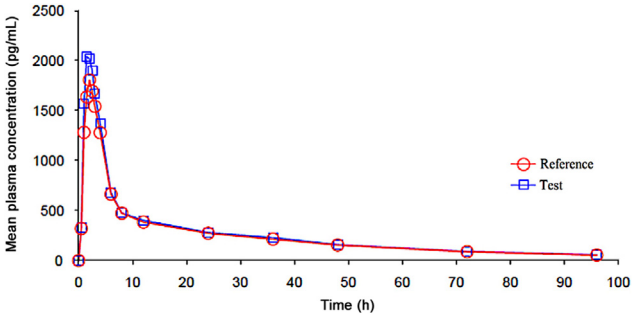

Fig. 3 Mean 17-desacetyl norgestimate plasma concentration–time profile following a 0.250 mg oral dose of norgestimate in human subjects.

Table 3 Stability results for 17-desacetyl norgestimate ($n=6$).

Stability	Spiked conc. (pg/mL)	Mean comparison sample conc. ±SD (pg/mL)	Mean stability sample conc. ±SD (pg/mL)	Change in stability (%)
Process[a]	60.085	57.192±1.812	59.158±1.921	3.44
	2036.767	2020.348±32.45	2051.736±9.247	1.55
	3916.86	3785.033±183.483	3914.996±102.645	3.43
Bench-top[b]	60.085	57.192±1.812	55.484±2.931	−2.99
	2036.767	2020.348±32.450	1987.116±44.628	−1.64
	3916.86	3785.033±183.483	3934.411±70.137	3.95
Freeze-thaw[c]	60.085	57.138±0.792	57.453±0.522	0.55
	2036.767	1959.264±9.407	1954.356±17.848	–0.25
	3916.86	3769.177±28.333	3740.842±19.078	−0.75
Freeze-thaw[d]	60.085	57.138±0.792	57.891±0.352	1.32
	2036.767	1959.264±9.407	1958.206±17.378	−0.05
	3916.86	3769.177±28.333	3767.058±27.159	−0.06
Long-term[e]	60.085	61.687±0.411	61.111±0.920	−1.32
	2036.767	2102.625±11.277	2109.831±9.024	1.68
	3916.86	4005.311±45.027	3970.363±43.975	0.45
Long-term[f]	60.085	61.687±0.411	61.496±0.507	−0.70
	2036.767	2102.625±11.277	2126.586±17.402	2.48
	3916.86	4005.311±45.027	3952.226±19.215	−0.01

[a]After 60 h in autosampler at 10 °C.
[b]After 6 h at room temperature
[c]After four freeze-thaw cycles at –30 °C.
[d]After four freeze-thaw cycles at –75 °C.
[e]–30 °C for 116 days.
[f]–75 °C for 116 days.

Table 4 Matrix effect for 17-desacetyl norgestimate and 17-desacetyl norgestimate D6 ($n=8$).

Parameter	LQC		MQC		HQC	
	Mean matrix factor±SD	RSD (%)	Mean matrix factor±SD	RSD (%)	Mean matrix factor±SD	RSD (%)
Analyte area	1.001±0.055	5.49	0.776±0.020	2.58	0.844±0.046	5.45
IS area	1.029±0.036	3.50	0.827±0.030	3.63	0.919±0.028	3.25
IS normalized	0.972±0.038	3.91	0.938±0.020	2.13	0.919±0.044	4.79

Table 5 Pharmacokinetic parameter.

Drug	Statistics	C_{max} (pg/mL)	t_{max} (h)	AUC_{0-t} (pg h/mL)	$AUC_{0-\infty}$ (pg h/mL)	K_{el} (h^{-1})	$t_{1/2}$ (h)
Test (T)	Mean	2289.2065	2.146	25,087.6725	28,025.4948	0.0230	31.896
	SD	538.8891	0.818	4382.7754	5186.3281	0.0060	7.381
	RSD (%)	23.54	38.14	17.47	18.51	26.10	23.14
Reference (R)	Mean	1979.9418	2.143	23,667.5552	26,326.6297	0.0230	31.819
	SD	454.1852	0.743	3868.6896	4031.8116	0.0054	7.832
	RSD (%)	22.94	34.69	16.35	15.31	23.55	24.61

C_{max}, the maximum plasma concentration.
t_{max}, the time to reach C_{max}.
AUC_{0-t}, the area under the plasma concentration–time curve from time zero to the last sampling time.
$AUC_{0-\infty}$, the area under the plasma concentration–time curve from time zero to infinity.
K_{el}, elimination rate constant.
$t_{1/2}$, elimination half-life.

conclude that processed samples were stable at least for 60 h at 10 °C in autosampler of the UPLC system. Results of bench-top stability for 17-desacetyl norgestimate were found to be stable for at least 6 h at room temperature in plasma samples. Freeze and thaw stability results indicated that the repeated freezing–thawing (four cycles) did not affect the stability of 17-desacetyl norgestimate for samples stored at −30 and −75 °C temperatures. Long-term stability of 17-desacetyl norgestimate in plasma was performed at LQC, MQC and HQC levels and was found to be stable for at least 116 days at −30 and −75 °C.

3.8. *Matrix effect*

The matrix factor for 17-desacetyl norgestimate and 17-desacetyl norgestimate D6 was calculated by comparing the area response for the analyte, IS and IS normalized area ratio observed in post-spiked samples with that of unextracted derivatized samples at LQC, MQC and HQC levels and the matrix effect was evaluated from the %RSD of mean matrix factor at each level. Three quality control samples at each level were analyzed and the %RSD of the samples analyzed was found ≤ 5.49% for analyte area, IS area and IS normalized area ratio for 17-desacetyl norgestimate and 17-desacetyl norgestimate D6 (Table 4).

Hence, this clearly proved that the elution of endogenous matrix peaks during the run had no effect on the quantification of 17-desacetyl norgestimate. Therefore, the method of extraction of 17-desacetyl norgestimate from plasma was robust enough and gave accurate and consistent results when applied to real subject samples.

3.9. *Application of method*

The proposed validated method was applied for the determination of 17-desacetyl norgestimate in plasma samples from ongoing projects for the development of an oral contraceptive formulation. Plasma samples were periodically collected up to 96 h after a single oral dose administration of a norgestimate and ethinyl estradiol 0.250 mg/0.035 mg tablet to 35 healthy female volunteers in each phase. The time periods at which the plasma samples were drawn were 0.00, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 4.00, 6.00, 8.00, 12.00, 24.00, 36.00, 48.00, 72.00 and 96.00 h after a single oral dose administration. A total of 1120 human plasma samples from 35 female volunteers were analyzed along with calibration standards and QC samples. Twelve calibration curves were made for sample quantification with forty-eight sets of interspersed LQC, MQC and HQC samples. No significant interference peak was found in predose samples for all volunteers. The mean (±SD) plasma maximum concentrations (C_{max}) obtained for the 17-desacetyl norgestimate test and reference formulations were 2289.2065 (±538.8891) pg/mL and 1979.9418 (±454.1852) pg/mL, respectively. The mean 17-desacetyl norgestimate plasma concentration–time profile following a 0.250 mg oral dose of norgestimate under fasting conditions to human subjects is shown in Fig. 3 and the pharmacokinetic data are presented in Table 5.

4. Conclusions

A first of its kind of analytical method using LC–MS/MS system was developed for the determination of 17-desacetyl norgestimate in human plasma. The described method was a simple, specific, rapid, reproducible and sensitive method with an LOQ of 20 pg/mL for 17-desacetyl norgestimate.

It was concluded that this sensitive and specific method was applicable for the quantitative determination of 17-desacetyl norgestimate in human plasma in pharmacokinetic and bioavailability studies of 17-desacetyl norgestimate.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jpha.2014.09.004.

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