

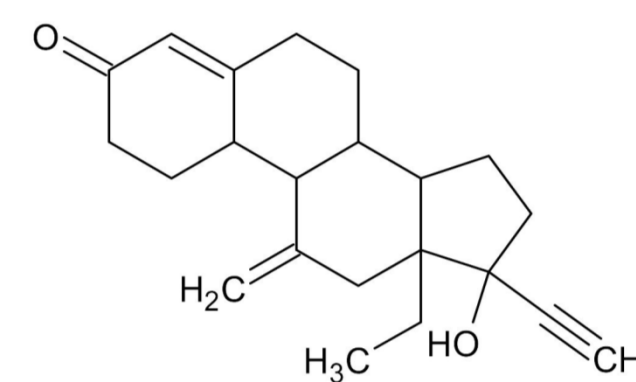


# Interference From Donor Gender on Long-Term Stability of Etonogestrel in Human Plasma

Nicolas Jean, Marie-Claude Th  berge, Sylvain Lachance, Nadine Boudreau, Ann L  vesque (inVentiv Health Clinical, Qu  bec, Canada)

## Introduction

Etonogestrel is the active metabolite of the inactive prodrug desogestrel, a steroidal progestin used in hormonal contraceptives. A bioanalytical study was performed for the determination of etonogestrel in human plasma from a clinical study. To cover the life of the study samples, the long-term stability (LTS) in human plasma of etonogestrel needed to be extended from 114 to 174 days at -20   C. However, LTS evaluation of 139, 183 and 196 days failed with %bias above 15% at the low concentration level for the stability samples.



Etonogestrel  
(3-keto-Desogestrel)  
MW: 324.46

## Method

Etonogestrel was validated over the range of 25-5000 pg/mL in human EDTA K<sub>3</sub> plasma. Etonogestrel is extracted from human plasma by liquid-liquid extraction. Analysis is performed on an API 5000 using reverse phase chromatography.

### Extraction Procedure

- Internal Standard: Etonogestrel-d<sub>6</sub>
- Sample Volume: 300   L
- Extraction Type: Liquid-liquid extraction followed by a derivatization of impurities
- Concentration factor: 3

### LC-MS/MS Analysis Old Conditions

Chromatographic mode: Reversed Phase  
 Analytical Column: Zorbax SB-C18 50 x 4.6mm  
 Column Temperature: 45   C  
 Elution mode: Isocratic  
 Mobile Phase A: Milli-Q Type Water/Methanol (30/70), Ammonium formate 1mM, Formic Acid 0.1%  
 Flow Rate: 1.0 mL/minute  
 Retention Times: 2.32 minutes  
 Acquisition time: 5.00 minutes  
 Detector: AB Sciex API 5000  
 Source: TurbolonSpray, Positive mode  
 Masses: 325→257 amu

### LC-MS/MS Analysis New Conditions

Chromatographic mode: Reversed Phase  
 Analytical Column: ACE Excel2 C18-PFP 50 x 3mm  
 Column Temperature: 25   C  
 Elution mode: Isocratic with column flush  
 Mobile Phase A: Milli-Q Type Water/Methanol (30/70), Ammonium formate 2mM, Formic Acid 0.1%  
 Flow Rate: 0.5 mL/minute  
 Retention Times: 1.91 minutes  
 Acquisition time: 4.50 minutes  
 Detector: AB Sciex API 5000  
 Source: TurbolonSpray, Positive mode  
 Masses: 325→257 amu

## Results

LTS was already validated after 114 days in human EDTA K<sub>3</sub> plasma and for different other durations in human EDTA K<sub>2</sub> plasma (Table 1). Evaluation after 139, 183 and 196 days gave positive biases up to 86% (Table 2). Stability issue was unlikely since biases were positive. A peak near the retention time of etonogestrel was observed in stability samples and its area increased overtime (Figures 1 and 2). This interfering peak was not observed in time=0 samples. The investigation lead to the hypothesis that the interfering peak was due to the gender of the matrix donor, specifically male donor since the failing tests out of criteria were done using plasma from both male and female donor pools. It was observed that the already proven 114 days LTS was evaluated with female donor only (Figure 3). New chromatography conditions were developed and showed that the interfering peak was indeed related to male donors. LTS evaluated with the new conditions showed that etonogestrel is stable over 362 days at -20   C in male and female pool of plasma (Figure 4 and Table 3).

Table 1. Long-Term Stability of Etonogestrel Validated in Matrix

Number of days	Matrix	QC1 Stability Samples		QC3 Stability Samples	
		% Bias	% CV	% Bias	% CV
8	EDTA K <sub>3</sub> plasma	4.57	2.71	-0.84	1.17
6	EDTA K <sub>2</sub> plasma	0.91	1.40	-5.10	4.70
78	EDTA K <sub>2</sub> plasma	2.99	6.47	-4.51	5.39
114	EDTA K <sub>3</sub> plasma	0.34	5.17	-0.78	1.07
135	EDTA K <sub>2</sub> plasma	-5.25	5.99	3.38	2.89

Table 2. Long-Term Stability Evaluation in EDTA K<sub>3</sub> Plasma

Number of days	QC1 Stability Samples		QC3 Stability Samples	
	% Bias	% CV	% Bias	% CV
139	42.27	3.83	3.27	1.04
183	73.88	3.58	-1.17	0.65
196	86.02	2.58	-1.72	0.82

Table 3. Long-Term Stability in Human EDTA K<sub>3</sub> for 362 Days at -20  C with the Improved Chromatography Conditions

	QC1				QC3			
	75.30 pg/mL				3765.00 pg/mL			
	Comparison Samples		Stability Samples		Comparison Samples		Stability Samples	
Measured Conc. (pg/mL)	% Bias	Measured Conc. (pg/mL)	% Bias	Measured Conc. (pg/mL)	% Bias	Measured Conc. (pg/mL)	% Bias	
74.99	-0.41	99.61	32.28	3595.90	-4.49	4261.70	13.19	
66.37	-11.86	87.51	16.22	3647.47	-3.12	4444.32	18.04	
75.41	0.15	87.56	16.28	3716.57	-1.29	4125.90	9.59	
75.41	0.15	78.88	4.75	3792.90	0.74	3922.57	4.19	
73.35	-2.59	83.40	10.76	3641.65	-3.28	3812.53	1.26	
68.74	-8.71	70.72	-6.08	3692.97	-1.91	3814.81	1.32	
<b>Mean</b>	<b>72.378</b>	<b>-3.88</b>	<b>84.613</b>	<b>12.37</b>	<b>3681.243</b>	<b>-2.22</b>	<b>4063.638</b>	<b>7.93</b>
<b>SD (��)</b>	<b>3.8855</b>		<b>9.6886</b>		<b>69.0572</b>		<b>258.0289</b>	
<b>CV%</b>	<b>5.37</b>		<b>11.45</b>		<b>1.88</b>		<b>6.35</b>	

## Chromatography

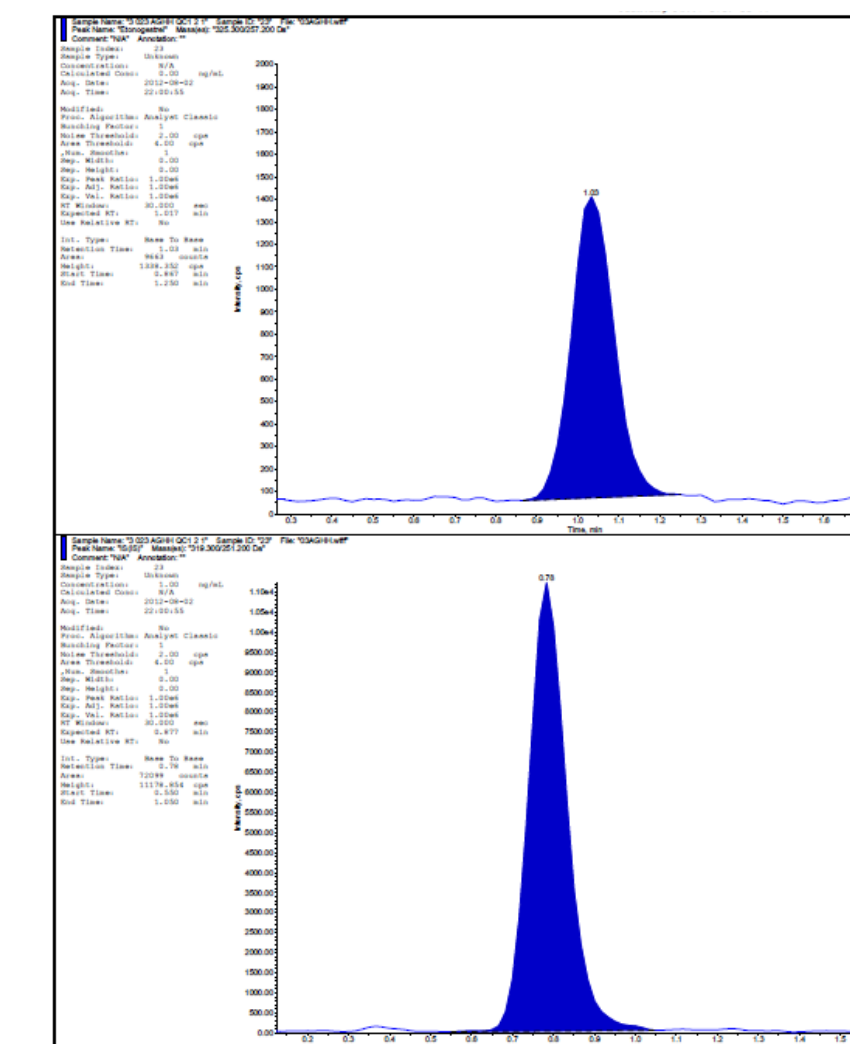


Figure 1. Representative Chromatogram of Low Quality Control in Mixed Plasma at T=0 (Comparison Sample). Retention Times are lower due to Cohesive System

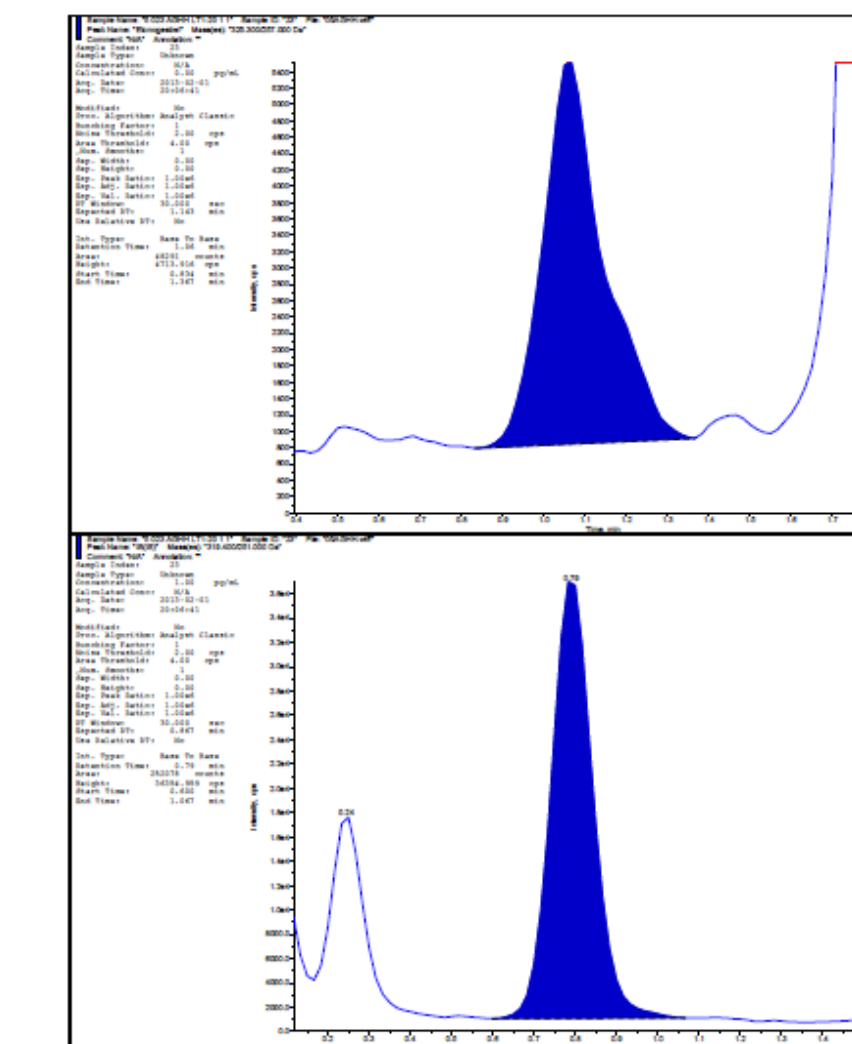


Figure 2. Representative Chromatogram of Low Quality Control in Mixed Plasma at T=183 days (Stability Sample). Retention Times are lower due to Cohesive System

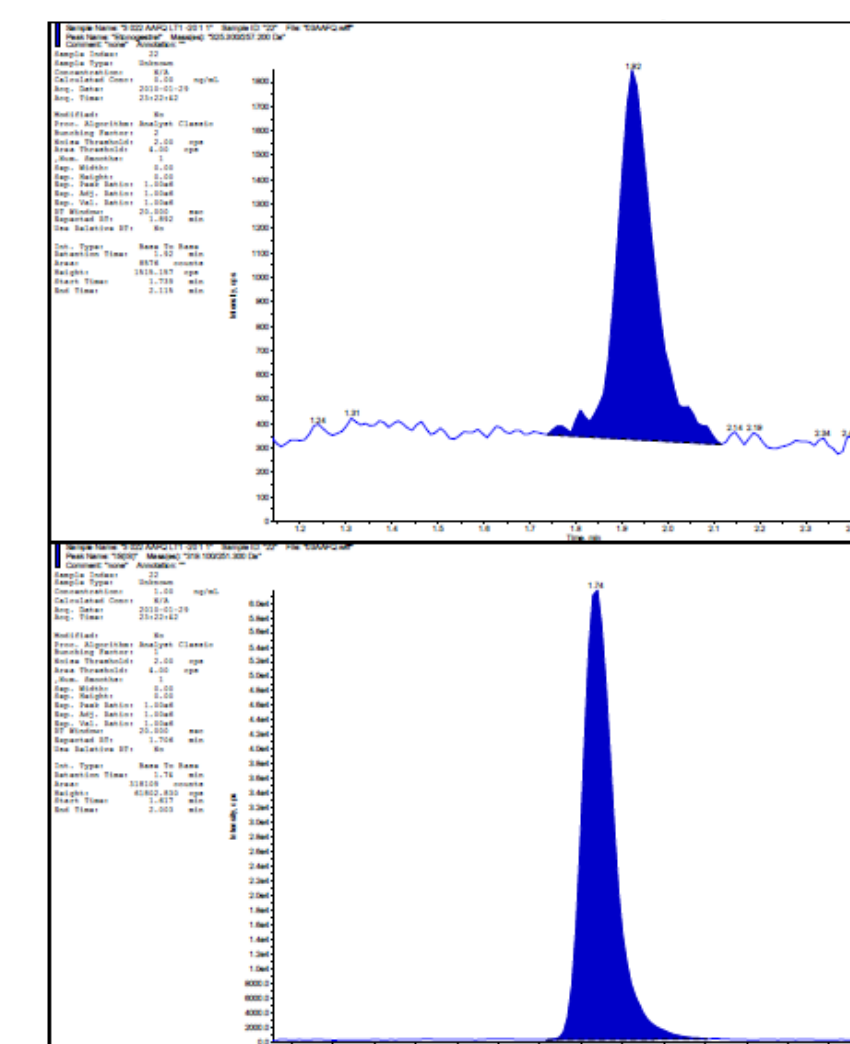


Figure 3. Representative Chromatogram of Low Quality Control containing only Female Plasma at T=114 days (Stability Sample). Retention Times are lower due to Cohesive System

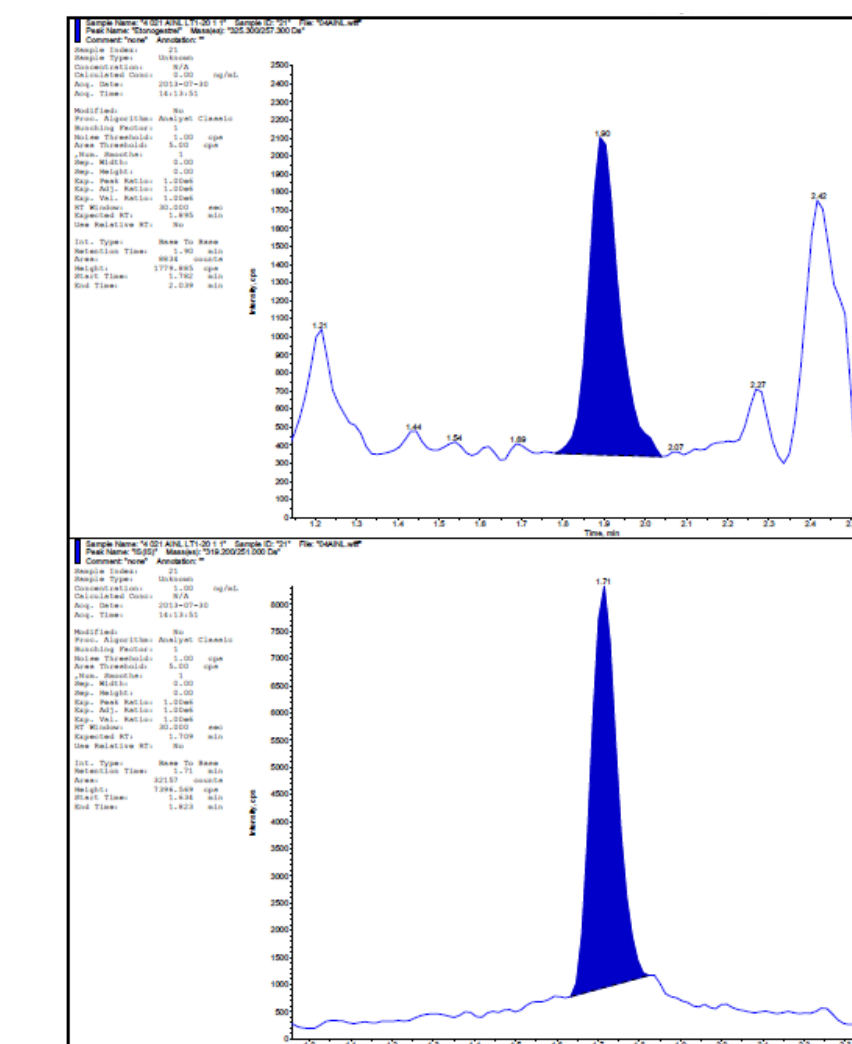


Figure 4. Representative Chromatogram of Low Quality Control in Mixed Plasma at T=362 days (Stability Sample)

## Conclusion

Male donor matrices produced an interfering peak overtime. New chromatographic conditions enable to separate this interference and evaluate the LTS. This has no impact on study samples analysis since only women were involved in the clinical study.

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