

Analyte	Calibration range (ng/mL)	Calibration curve Linear regression Equation	Coefficient of correlation	Retention time (min)	MRM Transition (m/z)	Collision energie (V)	Dwell Time (s)
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Anastrozole	1-100	$y=0.0134x+0.0004$	0.9995	1.27	294.20>225.20	-21.0	8.0
Letrozole	1-100	$y=0.0134x+0.0036$	0.9990	1.28	286.10>190.10	-15.0	8.0
Exemestane	1-100	$y=0.0043x+0.0033$	0.9993	1.80	297.20>121.10	-15.0	8.0
Fulvestrant	1-100	$y=0.0085x+0.0094$	0.9986	2.60	607.30>598.40	-19.0	8.0

Table 1. Calibration range, linear regression equation, coefficient of regression, time retention, MRM transition, dwell time and collision energy

Analyte	LLOQ				QC1				QC2				QC3				ULOQ			
	Intra day		Inter day		Intra day		Inter day		Intra day		Inter day		Intra day		Inter day		Intra day		Inter day	
	CV %	Biais %	CV %	Biais %	CV %	Biais %	CV %	Biais %	CV %	Biais %	CV %	Biais %	CV %	Biais %	CV %	Biais %	CV %	Biais %	CV %	Biais %
ANA	6.7	-5.4	7.6	1.4	3.5	-2.5	5.4	-7.9	2.5	-11.3	6.9	-11.1	2.0	0.1	6.4	-6.5	7.3	1.4	5.6	-4.6
LET	7.9	-4.9	10.1	-3.7	3.1	-0.9	9.1	7.2	4.7	3.7	5.3	-3.7	3.5	4.6	5.5	2.3	1.4	-0.2	4.4	4.4
EXE	7.7	11.3	12.1	4.6	6.4	-4.2	7.8	-4.6	6.9	-1.5	5.6	-1.5	7.4	2.9	7.4	-0.7	1.7	-3.5	6.6	1.5
FUL	18.5	-4.4	18.6	-2.0	8.0	2.8	10.6	-5.9	5.2	-1.1	6.6	-1.1	2.4	4.3	5.5	1.4	3.1	7.2	6.6	12.9

ANA: anastrozole ; LET: letrozole; EXE: Exemestane ; FUL: Fulvestrant

Table 2. Precision and accuracy inter and intraday of LLOQ, QC1, QC2, QC3, ULOQ

Anastrozole	Letrozole	Exemestane	Fulvestrant
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		CQ1	CQ3	CQ1	CQ3	CQ1	CQ3	CQ1	CQ3
Stability % true + CV (%)	Room temperature for 7 days	100.5±3.7	100.7±3.5	98.7±4.3	95.2±2.5	83.5±9.3	88.8±3.5	109.6±14	88.52±17.1
	4°C for 15 days	93.4±1.1	90.2±3.4	102.8±1.9	94.9±2.7	101.7±4.4	95.7±11.4	84.0±9.9	98.6±12.0
	- 20 °C for 30 days	97.6±1.4	97.1±0.2	98.4±7.3	92.2±1.4	98.8±5.6	96.7±3.4	114.9±5.8	107.4±2.5
	-20°C for 3 months	94.1±5.2	96.3±6.0	117.8±4.5	109.3±4.3	118.1±5.0	110.9±0.5	105.0±5.3	113.0±2.6
	Autosampler for 9 days	95.5±2.3	94.4±4.0	96.4±2.8	94.4±1.8	86.4±2.3	92.4±4.9	97.8±9.9	98.2±9.2
	After 3 freeze/thaw cycles	101.2±4.6	99.0±0.8	101.1±5.1	87.6±5.5	85.3±3.0	89.0±1.0	106.2±1.6	93.74±3.3

Table 3. Stability of the four analytes in different storage conditions

Analyte	Calibration standards (ng/mL)	1	10	25	50	75	100
Anastrozole	CV(%)	4.03	3.04	4.5	3.5	2.2	2.4
	Biais (%)	3.5	-4.4	0.9	-0.7	0.5	0.2
Letrozole	CV (%)	10.5	8.9	5.7	4.6	3.1	4.1
	Biais (%)	-0.7	-2.3	2.7	3.1	-0.7	-0.4
Exemestane	CV (%)	4.5	6.7	3.7	2.1	1.5	1.6
	Biais(%)	2.2	-3.6	0.09	-1.1	-2.3	2.3
Fulvestrant	CV (%)	9.5	6.7	4.8	4.8	3.6	5.1
	Biais(%)	7.02	-4.7	-1.3	-3.6	0.4	2.2

Table 4. Calibration standards (ng/mL) precision and accuracy over six different calibration curves

		Anastrozole	Letrozole	Exemestane	Fulvestrant
Mean matrix effect % true + CV (%)	CQ1	96.4±7.4	96.8±6.6	102.4±14.8	101.8±11.5
	CQ3	102.2±6.3	101.2±6.7	100.2±5.6	99.4±7.5
Hemolysis effect % true + CV (%)	CQ1	99.0±2.8	105.3±3.8	88.4±4.43	93.1±7.3
	CQ3	97.5±3.6	99.3±6.0	94.2±2.9	88.3±3.7
Extraction recovery % true + CV (%)	CQ1	91.8±4.8	98.9±11.7	76.2±6.6	87.4±4.0
	CQ2	99.1±0.5	84.6±13.4	79.7±3.2	97.8±31.3
	CQ3	87.5.1±3.9	92.7±3.5	77.4±2.6	83.0±7.8

Table 5. Matrix effect, hemolysis effect and extraction recovery

Dilution factor				
Analyte	1/5		1/2	
	Biais (%)	CV (%)	Biais (%)	CV (%)
Anastrozole	-7.9	3.6	9.5	2.8
Letrozole	-8.8	2.4	-5.7	4.7
Exemestane	-3.4	3.1	-0.3	6.7
Fulvestrant	5.3	7.2	2.0	5.0

Table 6. Dilution integrity

Analyte	Our method		Other methods		References
	Validated range	Transitions (m/z)	Validated range	Transitions (m/z)	
Anastrozole	0.4-150ng/mL	294.20>225.20	10-200ng/mL	294.3>225.20	M. van Nuland et al. 2019

Letrozole	0.3-1000 ng/mL	286.10>190.10	10-200ng/mL	286.2>217.1	M. van Nuland et al. 2019
Exemestane	0.8-200ng/mL	297.20>121.10	1.88-37.5ng/mL 0.4-40.0ng/mL	297.1>121.1 297 > 121	M. van Nuland et al. 2019 Wang LZ et al. 2015
Fulvestrant	1-400ng/mL	607.30>598.40	0.100 to 25.0 ng/mL	605.2 > 427.4	Alegete et al. 2017

Table 7. Comparison with other methods found in literature