#ThP18

Determination of Mifepristone in Human Plasma by Automated On-Line SPE Combined with MS/MS

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Introduction

Mifepristone is a synthetic steroid compound that has been studied in Phase II clinical trials for many medical uses, including: oral contraception, uterine fibroids, endometriosis, major depression with psychotic features, glaucoma, meningiomas, Cushing's syndrome, and various cancers. Mifepristone can be detected by LC/MS/MS following sample clean-up by various methods, including: protein precipitation, solid phase extraction, or liquid/liquid extraction. However, automated on-line SPE circumvents the need for an analytical HPLC column and provides a rapid, highly selective method of sample clean-up, allowing detection at levels relevant for therapeutic drug monitoring (100 ng/mL) in human plasma.

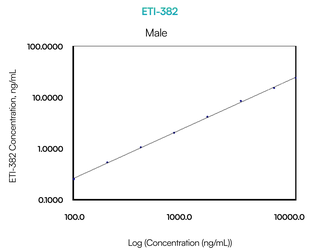
Methods

Instrumentation	Spark Holland Symbiosis SPE-LC system coupled to a Micromass Quattro Micro tandem quadrupole mass spectrometer
Analyte	Mifepristone
Internal Standard	Mifepristone-d_3
Sample Volume	0.05 mL
Sample Preparation Method	Plasma proteins are precipitated with 2 mL of 1% ammonium formate in methanol. The samples are vortexed, centrifuged, and a 0.7 mL aliquot is transferred to a well plate. A 0.005 mL aliquot was injected on the Symbiosis SPE-LC system.
Analytical Column	N/A
SPE Cartridge	HySphere C18 HD (Spark Holland, The Netherlands)
Mass Transitions	
Mifepristone:	430.30 > 372.20
I.S. (Mifepristone-d3):	433.30 > 375.20
On-line SPE Conditions	
Condition:	1 mL methanol
Equilibrate:	1 mL Water:Acetonitrile:Formic Acid (950:50:1, v/v/v)
Load:	1 mL Water:Acetonitrile:Formic Acid (950:50:1, v/v/v)
Wash:	0.5 mL Water:Acetonitrile:Ammonium Formate:Formic Acid (700:300:1.25:0.8, v/v/w/v)
Elute:	Standard mode (HPLC solvent stream is the eluting solvent) 0.45 mL Methanol:Water:Ammonium Formate:Formic Acid (700:300:0.75:0.6, v/w/v)
Retention Times	
Mifepristone, I.S.:	~0.4 min
Injection to Injection Cycle Time	2.03 minutes
Curve Range	100 to 10,000 ng/mL

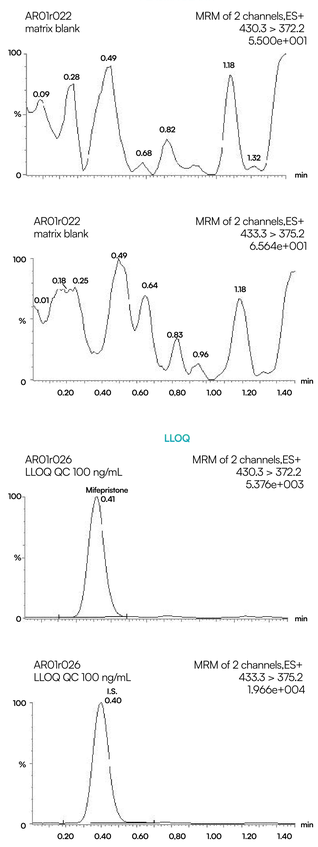
Conclusions

- A robust, specific, and simple assay for the analysis of mifepristone in human plasma has been validated.
- On-line SPE is an effective clean-up step to achieve specificity for mifepristone.
- The method is suitable to quantify human plasma in therapeutic drug monitoring studies.

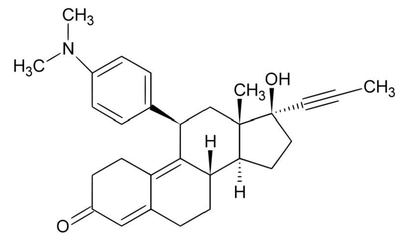
Representative Calibration Curve (100 to 10,000 ng/mL) for Mifepristone in Human Plasma



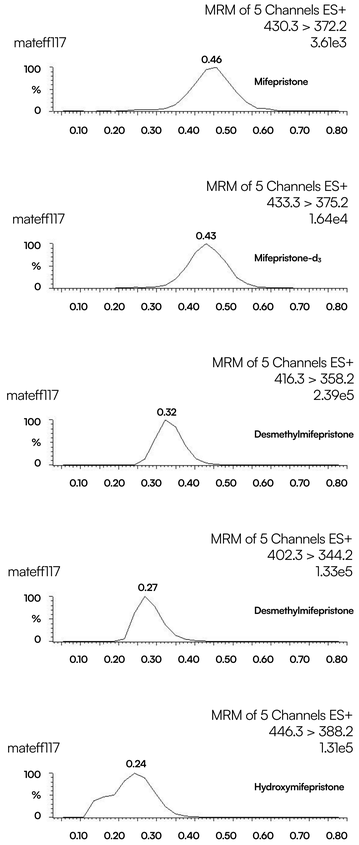
Representative Chromatograms for Mifepristone and Internal Standard in Human Plasma



Structure



Representative Chromatograms Showing Separation of Mifepristone and its Major Metabolites (Not Analyzed) on the SPE Cartridge



Accuracy and Precision

Interday

Analytical Run	Replicate	Concentration (ng/mL)				
		100	300	1,000	3,000	8,000
AR01	1	104	325	1,040	3,250	7,920
	2	104	311	1,060	3,110	8,010
	3	104	313	1,040	3,110	8,020
	4	107	308	1,040	3,150	8,070
	5	102	308	1,030	3,190	7,800
	6	106	306	1,070	3,190	7,890
AR02	1	99.8	299	979	3,090	7,650
	2	103	315	1,060	3,130	7,730
	3	102	306	1,020	3,160	7,660
	4	101	296	1,000	3,090	7,710
	5	98.3	305	989	2,980	7,600
	6	98.5	296	988	3,080	7,860
AR03	1	102	294	1,030	2,980	7,530
	2	95.0	307	1,020	3,160	8,030
	3	107	329	1,020	3,100	8,020
	4	105	291	1,050	3,180	7,830
	5	91.7	314	1,050	3,160	7,840
	6	110	311	1,020	3,040	7,450
AR04	1	97.7	327	992	3,090	7,890
	2	101	297	1,060	3,150	7,940
	3	102	315	1,000	3,000	7,750
	4	108	322	1,000	2,970	7,880
	5	99.9	301	1,020	3,050	7,860
	6	104	294	1,060	2,970	8,100
	Mean	102	308	1,030	3,100	7,840
	%CV	4.09	3.52	2.63	2.53	2.21
	%DEV	2.00	2.67	3.00	3.33	-2.00

Interday accuracy and precision were determined by analyzing replicate QC samples in human plasma at five concentrations over the course of four separate analyses.

Impact of Matrix Effect

Theoretical Concentration (ng/mL)	Plasma Lot Number	Replicate	Reported Concentration (ng/mL)	%DEV	Lot Acceptance
100	BC11810PM2	1	99.3	-0.700	Pass
		2	95.6	-4.40	Pass
		3	95.2	-4.80	Pass
	BC11810PM3	1	97.2	-2.80	Pass
		2	101	1.00	Pass
		3	98.2	-1.80	Pass
	BC11810PM4	1	95.8	-4.20	Pass
		2	97.3	-2.70	Pass
		3	95.1	-4.90	Pass
	BC11810PM6	1	92.6	-7.40	Pass
		2	93.5	-6.50	Pass
		3	91.6	-8.40	Pass
	BC040710PM1	1	95.5	-4.50	Pass
		2	97.5	-2.50	Pass
		3	91.5	-8.50	Pass
	BC040710PM3	1	97.1	-2.90	Pass
		2	98.1	-1.90	Pass
		3	104	4.00	Pass
				Overall Acceptance:	**100%**

The impact matrix effect on mifepristone was determined by calculating the %DEV of three replicates for each lot spiked at the LLOQ

HPLC Carry-Over Evaluation for Mifepristone in Human Plasma

Analytical Run	Lowest LLOQ Peak Height	Replicate	Concentration (ng/mL)		Carry-Over Percentage of LLOQ Peak Height*
			Carry-Over ULOQ	Carry-Over Peak Height	
MB01	3,813	1	397,797	0	0.00%
		2	406,438	0	0.00%
		3	449,103	0	0.00%
		4	429,762	0	0.00%
		5	428,220	0	0.00%
		6	508,032	0	0.00%

* Following injection of a unique ULOQ sample

HPLC carryover was evaluated by injecting a precipitation solution blank sample immediately following a unique ULOQ sample not included in the calibration curve. A mean response less than 20.0% of the LLOQ peak height indicates that there is no significant carry-over effect between injections.