Determination of Gestodene in Human Plasma by SLE-LC-MS/MS Using a Solid Core HPLC Column

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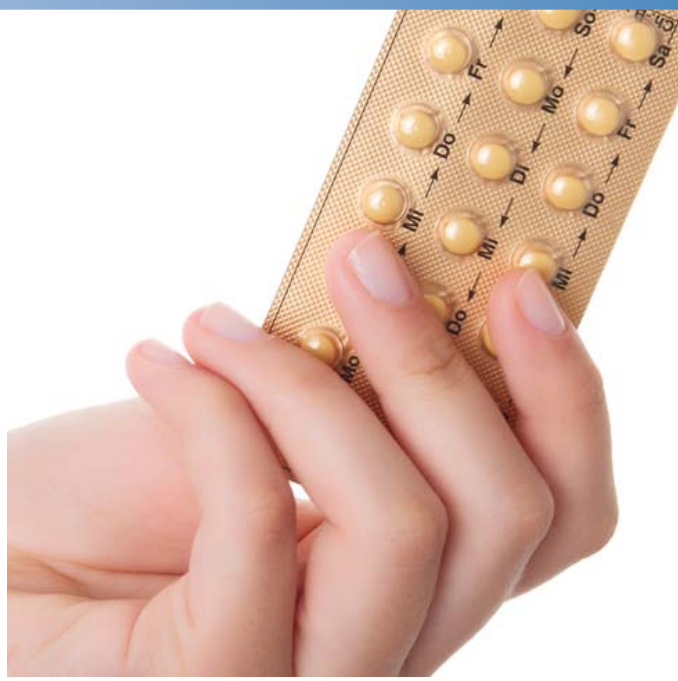
Key Words
HyperSep SLE, Accucore C18, solid core, gestodene

Abstract
A simple, rapid, and sensitive procedure for the determination of gestodene in human plasma by liquid chromatography-tandem mass spectrometry was developed and evaluated. The drug was isolated from a plasma matrix using Thermo Scientific™ HyperSep™ SLE, and the components of the resultant extracts were separated on a Thermo Scientific™ Accucore™ C18 HPLC column under reversed-phase, gradient conditions. Detection was performed on a triple quadrupole mass spectrometer using positive polarity, heated electrospray ionisation (HESI) conditions operating in selected reaction monitoring (SRM) mode.

Deuterated gestodene was used as the internal standard. Good chromatographic peak shape and linearity over the dynamic range 0.05 to 5 ng/mL was achieved with excellent precision.



Introduction
Gestodene is a synthetic form of progesterone that is used in oral contraceptives in combination with a synthetic estrogen.

The purpose of this study is to demonstrate the effectiveness of HyperSep SLE and an Accucore C18 HPLC column for the determination of gestodene in human plasma by liquid chromatography-tandem mass spectrometry using deuterated gestodene (d6 –gestodene) as an internal standard. Figure 1 shows the structure of gestodene.

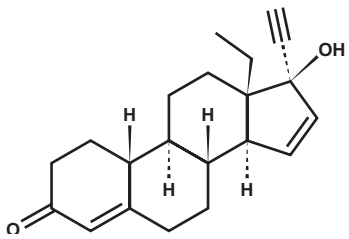


Figure 1: Structure of gestodene

Solid-supported liquid-liquid extraction (SLE) provides higher recovery and reproducibility compared to conventional liquid-liquid extraction (LLE) in combination with a robust and easy to use format that is amenable to high throughput analysis. This is achieved while maintaining the ability to provide clean extracts free of matrix interferences, such as phospholipids. This provides improved quality of results, a more efficient workflow, and ultimately reduced cost to the customer. SLE is useful for the extraction of components of moderate to low polarity (log P >2) and has several advantages over LLE including:

- It uses less solvent.
- It does not produce emulsification.
- It is easily automated using standard SPE processing equipment, for example, positive and negative pressure manifolds.
- It takes less time.
- It facilitates the use of small sample volumes.
- It eliminates problems associated with manual handling errors and cross contamination.

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SLE uses a solid packed bed of diatomaceous earth to support an aqueous sample. The aqueous sample is first loaded onto the support and allowed to adsorb. Small volumes of extraction solvent are then passed through the packing to allow the analytes to partition into the organic phase. This can be carried out several times, and the individual extraction volumes can be combined and dried down before being reconstituted and analyzed.

Accucore HPLC columns use Core Enhanced Technology™ to facilitate fast and highly efficient separations. The 2.6 µm diameter particles are not totally porous but have a solid core and a porous outer layer. The optimized phase bonding creates a series of high-coverage, robust phases. This coverage results in a significant reduction in secondary interactions and thus highly efficient peaks with very low tailing.

Experimental Details

Consumables	Part Number
Fisher Scientific™ Optima LC/MS grade methanol	A456-1
Water, from Thermo Scientific TKA Water Purification System	
Formic acid	
Gestodene and d6-Gestodene, kindly supplied by Accutest	
Human plasma with EDTA K2	
Thermo Scientific Micro+ Vial 300 µL, Fused Insert	60180-507
Thermo Scientific 9 mm Screw Top Closure w/PTFE/Silicone septum	60180-516

Sample Handling Equipment	Part Number
Thermo Scientific FinnPipette™ (100–1000 µL)	4642090
Thermo Scientific FinnPipette (10–100 µL)	4642070
Thermo Scientific FinnPipette (1–10 µL)	4642040

Solid Phase Extraction	Part Number
Thermo Scientific HyperSep SLE 500 mg / 3 mL	60109-500-3-7

Sample Preparation

Calibration standards

A stock solution of gestodene was prepared in methanol at a concentration of 0.2 mg/mL. Secondary standards (SS1–SS8) were prepared by subsequent serial dilution of the gestodene stock solution in methanol / water (80:20 v/v).

A stock solution of the internal standard, d6-gestodene, was prepared in methanol at a concentration of 0.1 mg/mL. Further dilutions were prepared in methanol / water (80:20 v/v).

Plasma spiked calibration standards of gestodene were prepared at eight different concentration levels (0.05, 0.1, 0.5, 1, 2.5, 3.5, 4.5, and 5 ng/mL) by fortification of plasma (285 µL) with 15 µL of appropriate stock standard. The internal standard (d6-gestodene, 20 µL) was added at the 100 ng/mL level into each of the calibrants. Standards S1 and S8 were prepared in duplicate while the remaining standards (S2–S7) were prepared singly. The concentration range selected was determined by targeting a lower limit of quantification at 0.05 ng/mL (equivalent to 5 half lives of C_{max}, 2.29 ng/mL). [1]

QC samples

Further samples were prepared to allow for calculation of precision. A mid-range concentration was prepared six times and subjected to the same extraction procedure to determine precision.

Extraction procedure for gestodene using HyperSep SLE

Pre-treatment:	300 µL of fortified plasma + 20 µL IS + 300 µL 0.1% formic acid in water
Application:	Add pre-treated sample to a HyperSep SLE cartridge.
Adsorption:	Using vacuum or positive pressure manifold, gently draw the plasma into the cartridge and leave to adsorb (5 min).
Elution:	Extract with 2 X 1000 µL methyl tert-butyl ether under gravity.

The eluents were combined and evaporated to dryness under a stream of nitrogen at 50 °C and reconstituted in methanol / water (80:20 v/v) (200 µL).

Separation Conditions		Part Number
Instrumentation:	Thermo Scientific Accela™ 1250 pump, Thermo Scientific Accela Open Autosampler	
Column:	Thermo Scientific Accucore C18 2.6 µm, 50 mm x 2.1 mm	17126-052130
Mobile phase A:	Water + 0.1% formic acid	
Mobile phase B:	Methanol + 0.1% formic acid	
Gradient:	Time (min) % B	
	0 5	
	0.2 5	
	1.0 60	
	3.0 60	
	3.2 95	
	3.8 95	
	4.0 5	
	5.0 5	
Flow rate:	0.4 mL/min	
Column temperature:	40 °C	
Detection:	MS	
Injection volume:	10 µL	
Syringe volume:	100 µL	
Loop size:	20 µL	
Syringe flush:	Wash1: water/methanol (80:20 v/v)	
	Wash2: acetonitrile/acetone/water (90:10:10 v/v)	
Cool Stack temperature:	10 °C	
Detection:	MS	
Column backpressure:	145 bar	
Run time:	5.0 minutes	

Mass Spectrometry Conditions	
Instrumentation:	Thermo Scientific TSQ Vantage™ triple-stage quadrupole mass spectrometer
Ion source type:	HESI-II
Polarity:	Positive
Spray voltage:	3500 V
Vaporizer temperature:	400 °C
Sheath gas pressure:	50 arb
Ion sweep gas pressure:	0 arb
Auxiliary gas pressure:	10 arb
Capillary temperature:	375 °C
Declustering voltage:	0 V
Collision pressure:	1.5 mTorr

MS acquisition parameters

Quantification was performed by selected reaction monitoring (SRM) using the precursor-to-product combinations shown in Table 1.

Compound	Precursor *m/z*	Product *m/z*	Collision energy	S-Lens
Gestodene	311.2	109.1	25	79
D6-gestodene	317.2	114.1	27	79

Table 1: TSQ Vantage MS acquisition parameters

Scan type:	SRM
Q1 peak width:	1.2 (FWHM)
Q3 peak width:	1.2 (FWHM)
Scan width:	0.02 *m/z*
Scan time:	0.1 s
MS acquisition time:	5.0 min

Data Processing

All data was processed using Thermo Scientific LCQuan (v. 2.6) software. The algorithm for integration was ICIS.

Results

The retention time of gestodene was 2.84 minutes.

Linearity of response

A graphical plot of relative response as a function of the concentration of gestodene is shown in Figure 2. The calibration data is summarized in Table 2.

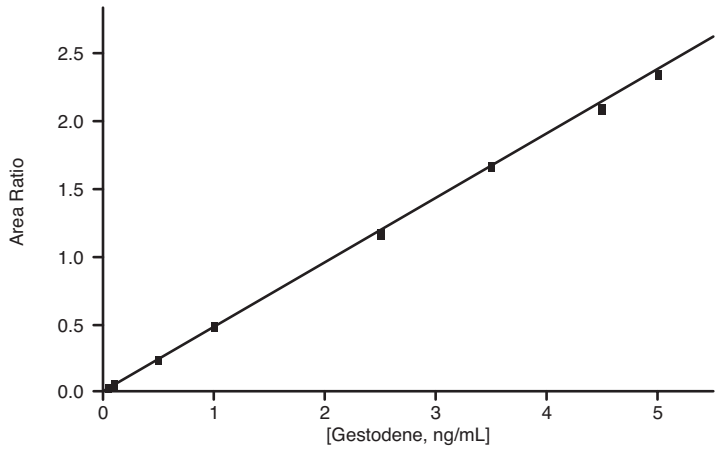

Figure 2: Linearity of response over the dynamic range 0.05–5 ng/mL

Standard Ref.	Nominal concentration [gestodene] ng/mL	Calculated concentration [gestodene] ng/mL	Relative error %
S1	0.05	0.048	-3.6
S2	0.1	0.105	4.8
S3	0.5	0.483	-3.4
S4	1.0	1.02	2.3
S5	2.5	2.48	-0.7
S6	3.5	3.55	1.5
S7	4.5	4.46	-1.0
S8	5.0	5.00	0.0

Table 2: Linearity of response for the determination of gestodene in human plasma

The analytical response was found to be linear with a coefficient of determination (r^2) of 0.999 in the range 0.05 to 5 ng/mL.

Precision

For the mid-range concentration (2.5 ng/mL) extracted plasma samples, precision was excellent (Table 3).

Nominal Concentration (ng/mL)	No. of samples (N)	%CV	% difference
2.5	6	1.86	-4.51 to -0.29

Table 3: Precision results for the determination of gestodene in human plasma

Specificity and sensitivity

SRM chromatograms derived from the examination of the extracted blank plasma and extracted spiked plasma samples are shown in Figure 3.

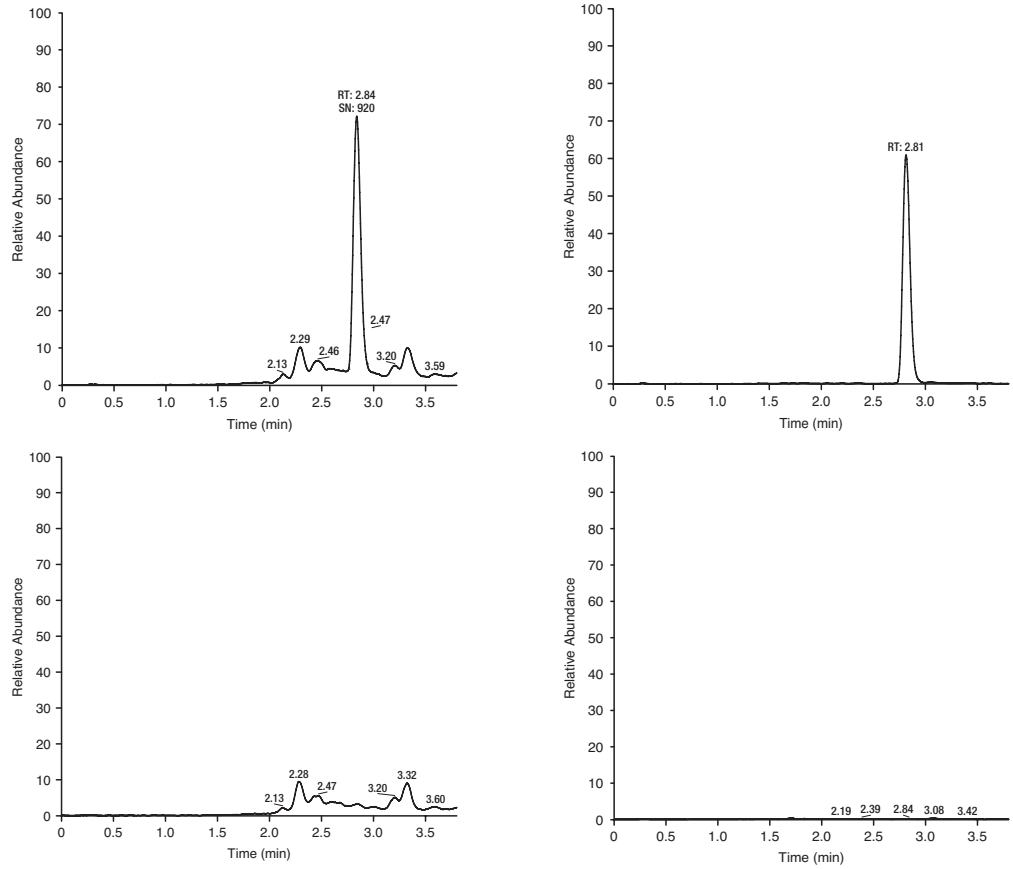

Figure 3: Representative chromatograms of gestodene SRM, extracted from human plasma (at 2.5 ng/mL top left, blank bottom left) and deuterated gestodene IS (top right, blank bottom right).

Conclusion

- An analytical procedure based upon SLE-LC-MS/MS for the determination of gestodene in human plasma was successfully developed and evaluated.
- The procedure was found to exhibit good linearity (r^2 = 0.999) for concentrations of gestodene in the range from 0.05 to 5 ng/mL. The accuracy and precision were found to be excellent and well within the limits of acceptance specified by the FDA. [2] The performance characteristics of the method combined with its simplicity and rapidity mean that it can be adopted routinely in bioanalytical environments.

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References

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