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Research Paper

A Novel Liquid Chromatography Tandem Mass Spectrometric Method for the Determination of Dienogest in Bulk and Commercial Formulation

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Abstract:
The present study was aimed to describe a novel, highly sensitive liquid chromatography coupled with mass spectrometry method for the determination of dienogest from the commercially available formulation. The chromatographic separation was achieved by C_{18} (50 mm×4.6, 3 µ) column using the mobile phase 10 mM ammonium formate and methanol in the ratio of 10:90 v/v, using the flow rate of 0.5 ml/min. The calibration curve was plotted over the range of 0.6-90 ng/ml with a correlation coefficient (r^2) of 0.994. The accuracy of the method was found to be over the range of 96.0 to 101.56 %. The mass spectrometric method was validated as per the ICH guidelines. The presence of dienogest in the formulation was successfully quantified using the developed method.

Keywords: Dienogest, formulation, ICH, mass spectrometric, validation

INTRODUCTION

Dienogest is also known as (17α)-17-Hydroxy-3-oxo-19-norpregna-4,9-diene-21-nitrile and is a semisynthetic progestogen and also possesses the properties of 17α-hydroxyprogesterone with a molecular formula of $C_{20}H_{25}NO_2$ and molecular mass of 311.42 g/mol (fig. 1). It has antiandrogenic properties and a derivative of 19-nortestosterone and it is commonly marketed as 2 mg tablets with a brand name of Endoheal, Endofit, Dienofem, Dinogest, Dinofirst[1]. It is primarily used as a contraceptive in combination with ethinylestradiol or in other combination form pills.

A thorough literature review revealed that a reversed phase high-performance liquid chromatography method is reported for the detection and quantification of dienogest[2] and to best of our knowledge a liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the estimation of dienogest in human plasma is reported by which the developed method was less sensitive[3]. Hence, the present study was aimed to develop a novel highly sensitive LC-MS/MS method and estimate dienogest in bulk and in commercial formulation.

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MATERIALS AND METHODS

Chemicals and reagents:

Dienogest was obtained as a gift sample from Indian Pharmacopeia Commission, New Delhi. LC-MS grade methanol and acetonitrile were acquired from Sigma Aldrich. Formic acid and ammonium formate acquired from Rankem Fine Chemical Ltd. LC-MS grade water was procured from a Milli-Q RO system. Dienogest formulation has been procured from the local market, Bangalore.

Instrumentation:

A Shimatzu LC-MS 8030 system equipped with ESI interface, SPD-M20 PDA detector, LC-20AD pump, CBM-20 alite controller, CTO-20AC column oven, SIL-20AC Autosampler and monitored by Lab solution data station. The mass parameters which were optimized for the detection of dienogest is as follows: Desolvation line (DL) temperature was 250°, probe temperature was ambient, block temperature was set at 350°, CID gas

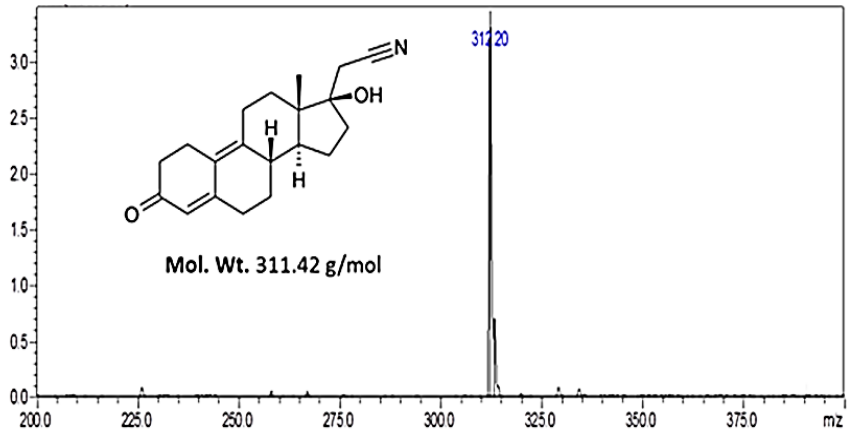

Fig. 1: Chemical structure and mass scan spectra of dienogest in positive ionization mode

maintained at 230 kPa, N_2 was used as nebulizing gas at a flow rate of 3 l/min and it was also used as drying gas at a flow rate of 15 l/min, respectively.

Preparation of standard solution and quality control (QC) samples:

About 10 mg of dienogest was dissolved in methanol and the solution was made up to 10 ml to produce a 1 mg/ml stock solution and was refrigerated below 8° until use. From the prepared stock solution, further dilutions were made to produce the linearity concentration of 0.6, 2.0, 7.0, 15, 25, 36, 45 and 90 ng/ml, respectively. Similarly, quality control samples were prepared by diluting the stock solution at three different concentration range of 2.0, 25 and 55 ng/ml (low, medium and high).

Method validation:

The method was validated for accuracy, precision, selectivity, linearity, recovery, detection limit and quantitation limit based on the principles of the ICH guidance[4].

Specificity is the ability of the method to measure the response of the analyte under the existence of some potential impurities and other excipients.

The linearity was plotted for concentration levels ranging from 0.6 to 90 ng/ml for dienogest. The linearity of the proposed method was evaluated using a calibration curve to calculate the correlation coefficient and slope.

For accuracy studies, samples of low quality control (LQC), medium quality control (MQC) and high quality control (HQC), equivalent to 2.0, 25 and 55 ng/ml, respectively were analyzed at six replicates. The precision studies were carried out as intra-day and inter-day precision studies at three QC levels and percent relative standard deviation (% RSD) of the regressed concentration was taken to report the precision of the method. The accuracy of the method was determined by regressed concentration represented as a percentage of the nominal concentration.

The detection limit was studied based on the signal-to-noise ratio of 3:1. The quantification limit was also studied based on the signal-to-noise ratio, where the drug can be quantified with a minimum peak area in the ratio of 10:1.

System suitability test plays an essential part in the method development process. The number of theoretical plates (N), retention time (Rt), resolution (Rs), peak asymmetric factor and tailing factor (T) were evaluated for three replicates.

The robustness of the methods was studied by changing the experimental conditions (the source of reagents and column type) and optimized conditions (mobile phase ratio, flow rate and pH).

RESULT AND DISCUSSION

The chromatographic and mass spectrometric method was optimized based on trial and error method by modifying the strength, pH and ratio of mobile phase to achieve symmetric peak. Similarly, the mass spectrometric parameters such as collision energy, the voltage applied, DL temperature and nebulizer gas flow were optimized to achieve a highly sensitive method.

Finally, the separation was achieved using a C_{18} (50 mm×4.6, 3 µ) analytical column using the mobile phase of methanol and 10 mM ammonium formate in the ratio of 10: 90, v/v at a flow of 0.5 ml/min. The mass spectrometric conditions optimized were; DL temperature of 250°, probe temperature was kept ambient, block temperature was set at 350°, CID gas maintained at 230 kPa, N_2 was used as nebulizing gas at a flow rate of 3 l/min and it was also used as drying gas at a flow rate of 15 l/min, respectively. Further, the

chromatograms were recorded and processed using Lab solution data station (fig. 2A).

The specificity test demonstrates that the used excipients did not interfere with the peak of the main compound. At the retention time of the drug dienogest no peaks were eluted (fig. 2B). Hence, the results prove that the method developed was selective for the detection of dienogest in the formulation.

The method was evaluated for linearity by six determinations at eight concentration levels with a range of 0.6-90 ng/ml for dienogest. A calibration curve of the method was found to be linear with a regression coefficient r^2 of 0.9941 as shown in fig. 3**.**

Accuracy and precision of the method were evaluated by calculating the intra-day and inter-day precision at three QC levels at six replicates. The mean accuracy level was determined by percentage nominal concentration and the mean precision level was found to be within the limits (≤ 15 %). The results indicate that the developed method was accurate and precise for the analysis (Table 1).

The detection limit was found to be 0.3 ng/ml based on the signal-to-noise ratio of 3:1. The presence of lower detection limit produced a highly sensitive quantitation limit of 0.6 ng/ml with a signal-to-noise ratio of 10:1.

For the estimation of dienogest from the tablets, twenty tablets were weighed and powdered. The weight equivalent to 10 mg was weighed accurately and transferred into 10 ml volumetric flask. Dissolve the content using 5 ml methanol and filter through a 0.45 µm filter and the final volume made up to mark with diluent methanol**.** The percentage recovery was calculated at the three QC levels (2.0, 25 and 55 ng/ml) and the percentage recovery was found to be 96.0 to 101.56 % (Table 2). Due to the good recovery of dienogest, this method was found to be simple and cost-effective.

The system suitability studies were performed by conducting experiments and examining the changes in the peak elution time (retention time), separation, peak asymmetric factor and tailing factor. The results were found to be within the limits and are summarized in Table 3**.**

Robustness and ruggedness studies conclude that no significant changes in the chromatographic parameters were observed when the experimental conditions (flow

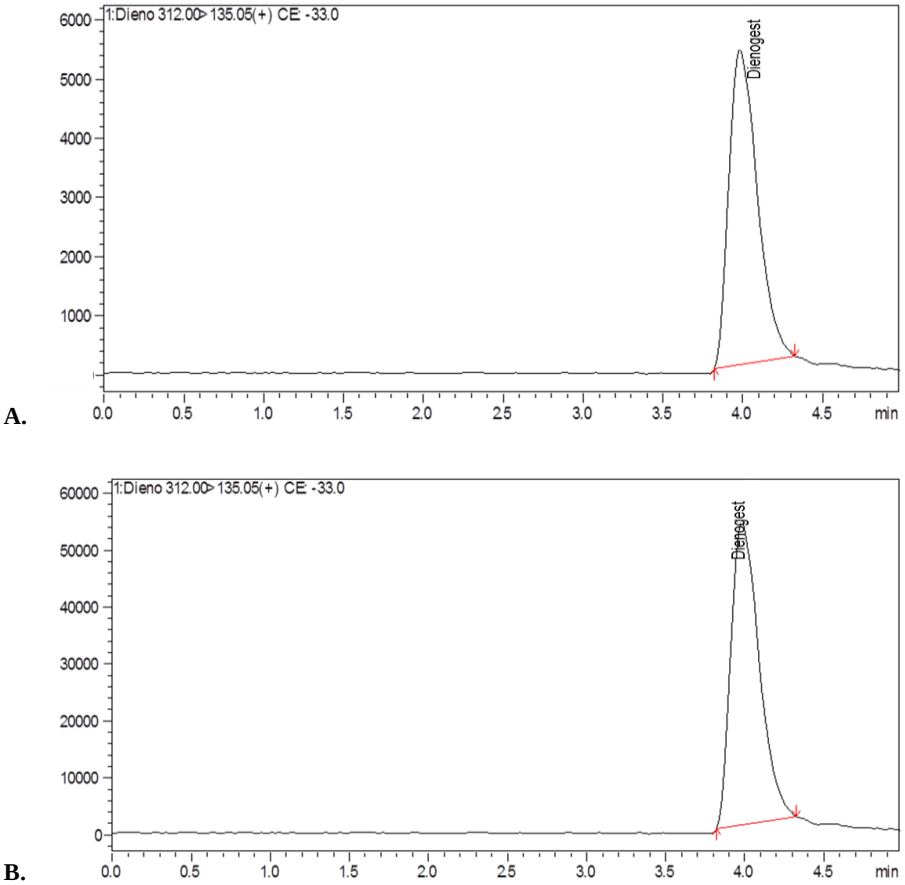

Fig. 2: (A) Standard MRM chromatogram of dienogest (2.0 ng/ml) and (B) sample MRM chromatogram of dienogest in commercial formulation (25 ng/ml)

TABLE 1: ACCURACY AND PRECISION RESULTS OF DIENOGEST

QC samples (ng/ml)	Conc. found (ng/ml) ±% RSD (n=3)	Intra day		Inter day	
		Accuracy	Precision (% RSD)	Accuracy	Precision (% RSD)
2.0	1.830±1.3	91.50	2.73	83.00	2.13
25	24.70±0.6	98.80	2.58	92.36	2.82
55	54.85±0.5	99.72	0.95	97.14	2.25

Data given in this table represents mean, n=3, RSD: relative standard deviation

TABLE 2: RECOVERY STUDIES FOR THE FORMULATION

Formulation*	Label claim	Assay amount (ng/ml)	Amount quantified ±% RSD	% Recovery
T_1	2 mg	2.0	1.920±0.31	96.00
		25	24.65±0.73	98.60
		55	55.86±0.13	101.56

Data given in this table represents mean, n=6, RSD: relative standard deviation. *Endoreg containing 2 mg of Dienogest

TABLE 3: SUMMARIZATION OF SYSTEM SUITABILITY PARAMETERS RESULTS

S.No	Parameters	Dienogest
1	Retention time (min)	3.981
2	Theoretical plates (N)	4568
3	Tailing factor	1.1
4	Asymmetric factor	0.9
5	Regression coefficient (r^2)	0.9941
6	Regression equation	y = 527.49x - 785.94
7	Linearity range	0.6 - 90 ng/ml
8	Detection limit (LOD)	0.3 ng/ml
9	Quantification limit (LOQ)	0.6 ng/ml

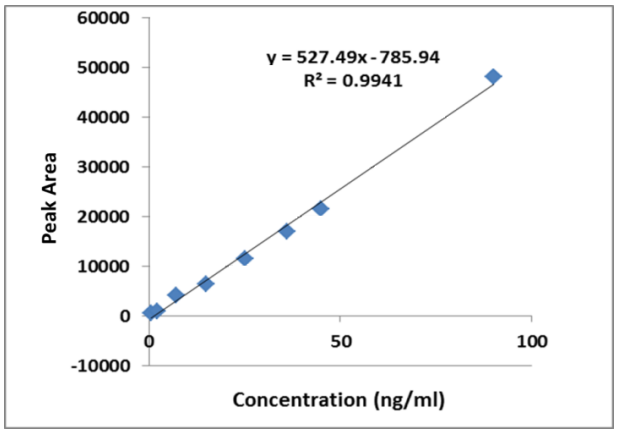

Fig. 3: Linearity range of dienogest at seven concentration levels (0.6-90 ng/ml)

rate, % methanol and pH) were changed, demonstrating that the method to be robust.

The developed method was found to be rapid, simple, precise, accurate and sensitive for the determination of dienogest and the method was validated as per the ICH guidelines, which were found to be stable under prescribed conditions. The developed method showed good linearity over the concentration range of 0.6 to 90 ng/ml. The accuracy of the developed ranged from 96.0 to 101.56 %. Hence, it was concluded that the developed method can be successfully applied for the quantitative determination of dienogest in pharmaceutical formulation and in bulk drug. The developed method LC-MS/MS can be used for the quantification of dienogest in plasma samples.

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Conflict of interest:

The authors declare that there are no conflicts of interest.

Financial interest/scholarship:

Nil.

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