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Research

Quantitative estimation of segesterone acetate and ethinyl estradiol in tablet dosage forms by rp-hplc method

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Abstract

A Rapid and Precise Reverse Phase High Performance Liquid Chromatographic method has been developed for the validated of Segesterone Acetate and Ethinyl Estradiol, in its pure form as well as in tablet dosage form. Chromatography was carried out on X-Terra C18 (4.6 x 150mm, 5µm) column using a mixture of Methanol: TEA Buffer pH 4.5: Acetonitrile (65:15:20) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 212 nm. The retention time of the Segesterone Acetate and Ethinyl Estradiol was 2.090, 5.289 ±0.02min respectively. The method produce linear responses in the concentration range of 5-25mg/ml of Segesterone Acetate and 45-225mg/ml of Ethinyl Estradiol. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Segesterone Acetate and Ethinyl Estradiol, RP-HPLC, validation.

INTRODUCTION

Analytical chemistry is the branch of chemistry involved in separating, identifying and determining the relative amounts of the components making up a sample of matter. It is mainly involved in the qualitative identification or detection of compounds and the quantitative measurement of the substances present in bulk and pharmaceutical preparation.

Measurements of physical properties of analytes such as conductivity, electrode potential, light absorption or emission, mass to charge ratio, and fluorescence, began to be used for quantitative analysis of variety of inorganic and biochemical analytes. Highly efficient chromatographic and electrophoretic techniques began to replace distillation, extraction and precipitation for the separation of components of complex mixtures prior to their qualitative or quantitative determination. These newer methods for separating and determining chemical species are known collectively as instrumental methods of analysis. Most of the instrumental methods fit into one of the three following categories viz spectroscopy, electrochemistry and chromatography

High Performance Liquid Chromatography

HPLC is a type of liquid chromatography that employs a liquid mobile phase and a very finely divided stationary phase. In order to obtain satisfactory flow rate liquid must be pressurized to a few thousands of pounds per square inch.

The rate of distribution of drugs between Stationary and mobile phase is controlled by diffusion process. If diffusion is minimized faster and effective separation can be achieved. The techniques of high performance liquid chromatography are so called because of its improved performance when compared to classical column chromatography advances in column chromatography into high speed,efficient ,accurate and highly resolved method of separation.

For the recent study metformin and Sitagliptin was selected for estimation of amount of analyte present in formulation and bulk drug. The HPLC method is selected in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low.

HPLC components

The essential components[4] of a complete HPLC system are solvent delivery system (Pump), detector, fixed volume injector loop or autosampler, solvent reservoirs, packed column, data system and recorder. A schematic of a simplified HPLC system is shown in above Figure.

Column

The column is probably the heart of HPLC system. The development of this column technology leads to the evolution of the HPLC instrumentation systems used today.The conventionally used HPLC columns are particle packed columns. The key of column selection when previous separation is not available resides in knowing the chemistry of the sample. Columns should never be dry. A dry column will eventually have voids because the packing will shrink away from the wall, which would result in band broadening. Before running a sample in HPLC the column should be equilibrated. Usually column equilibrium is achieved after passage of 10 – 20 column volumes of the new mobile phase through the column. Insufficient column equilibrium usually leads to retention difference.

Injector or Auto sampler

Samples are usually introduced by syringe injection via a manual injector into the mobile phase stream or by the use of an auto sampler. The important aspects in sample introduction are precise and reproducible injections. This is especially important with quantitative analysis where the reproducibility of the peak response is dependant on the precision of the sample introduction. Direct syringe injection through a manual injector was the first popular method of sample introduction. As HPLC instrumentation evolved, many auto sampler techniques were applied so that sample introduction has become more precise and rapid.

Detector

HPLC detectors include ultraviolet-visible, fluorescence, electrochemical, refractometer, mass spectrometer and others. The UV visible absorption detector is the most widely used detector in liquid chromatography, since most organic compounds show some useful absorption in the UV region. This detector is fairly universal in application, although sensitivity depends on how strongly the sample absorbs light at a particular wavelength.

Solvent reservoir

Different containers are used as a solvent delivery system reservoir. The best material from which the containers are made is glass. Plastic containers are not recommended as it leads to plasticizer leaching. The container should be covered to prevent solvent evaporation. The tubing from the reservoir can be made of stainless steel or Teflon, and both are satisfactory.

Data handling and analysis

Data handling in HPLC is as important to the success of any experiment or analysis as any other components in the system. It is part of good HPLC techniques to properly label and document the analytical results. The advanced computer softwares used now in data handling and analysis allow easy recording and storage of all chromatographic data.

MATERIALS AND METHOD

Segesterone Acetate-Sura labs, Ethinyl Estradiol (Pure)-Sura labs, Water and Methanol for HPLC-LICHROSOLV (MERCK), Acetonitrile for HPLC-Merck.

HPLC method development
Trails
Preparation of standard solution: Accurately weigh and transfer 10 mg of Segesterone Acetate and Ethinyl Estradiol working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.
Further pipette 2.25ml of the above Segesterone Acetate and 0.45ml of the Ethinyl Estradiol stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:
Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization: Initially the mobile phase tried was Methanol: Water, Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: TEA buffer pH 4.8 in proportion 32:68 v/v respectively.

Optimization of Column: The method was performed with various columns like C18 column, X- bridge column, Xterra. Phenomenex Gemini C18 (4.6mm×150mm, 5.0 µm) particle size was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions:

Instrument used	:	Waters HPLC with auto sampler and PDA Detector 996 model.
Column	:	Phenomenex Gemini C18 (4.6mm×150mm, 5.0 µm) particle size
Column temperature	:	38˚C
pH	:	4.8
Mobile phase	:	Methanol: TEA buffer pH 4.8 (32:68v/v)
Flow rate	:	1ml/min
Wavelength	:	248nm
Injection volume	:	20µl
Run time	:	7 min

Method validation
Preparation of mobile phase
Preparation of mobile phase: Accurately measured 320ml (32%) of HPLC Methanol and 680ml of TEA buffer (68%) were mixed and degassed in a digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.
Diluent Preparation: The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase	:	Methanol: TEA Buffer pH 4.5: Acetonitrile (65:15:20)
Column	:	X-Terra C18 (4.6×150mm, 5.0 µm)
Flow rate	:	1 ml/min
Wavelength	:	212 nm
Column temp	:	Ambient
Injection Volume	:	10 µl
Run time	:	10 minutes

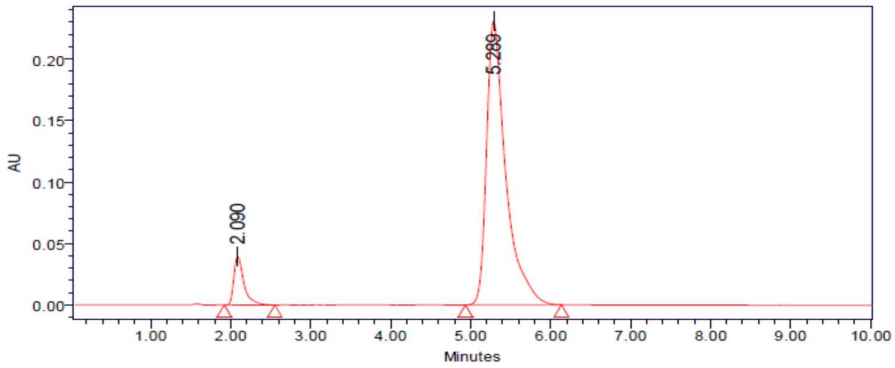

Fig 1: Optimized Chromatogram

Table 1: peak Results for optimized

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Segesterone Acetate	2.090	372127	39691		1.71	5588
2	Ethinyl Estradiol	5.289	3864999	231195	9.81	1.78	5699

From the above chromatogram it was observed that the Segesterone Acetate & Ethinyl Estradiol peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogeram (Sample)

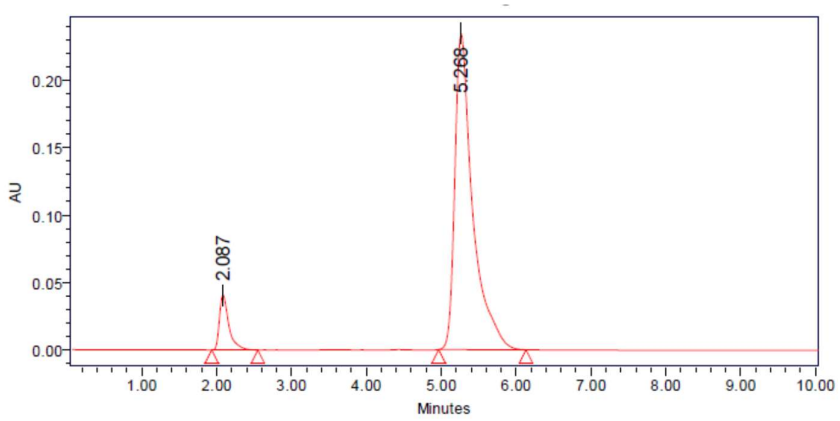

Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Segesterone Acetate	2.087	356548	41156		1.73	5556
2	Ethinyl Estradiol	5.268	3896494	234962	9.83	1.92	5805

- *Resolution between two drugs must be not less than 2*
- *Theoretical plates must be not less than 2000*
- *Tailing factor must be not less than 0.9 and not more than 2.*
- *It was found from above data that all the system suitability parameters for developed method wer within the limit.*

System Suitability

Table 3: Results of system suitability for Segesterone Acetate

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Segesterone Acetate	2.090	342127	39691	5464	1.42
2	Segesterone Acetate	2.090	342425	39692	5577	1.42
3	Segesterone Acetate	2.089	342563	39991	5099	1.44
4	Segesterone Acetate	2.089	347977	40397	5144	1.43
5	Segesterone Acetate	2.085	352915	40964	5675	1.47
Mean			345601.4			
Std. Dev			4757.233			
% RSD			1.376509			

- *%RSD of five different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is suitable.*

Table 4: Results of system suitability for Segesterone Acetate

S. no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Ethinyl Estradiol	5.289	3864999	231195	5787	1.46	9.80
2	Ethinyl Estradiol	5.289	3864997	232183	5909	1.47	9.81
3	Ethinyl Estradiol	5.338	3881444	231045	5488	1.48	9.81
4	Ethinyl Estradiol	5.327	3896953	231968	5033	1.40	9.83
5	Ethinyl Estradiol	5.262	3900104	233542	5388	1.43	9.82
Mean			3881699				
Std. Dev			16802.83				
% RSD			0.432873				

- *%RSD for sample should be NMT 2*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Assay (Standard)

Table 5: Peak results for assay standard

S no	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Segesterone Acetate	2.090	348127	39691		1.70	5588	1
2	Ethinyl Estradiol	5.289	3864999	231195	9.81	1.77	5629	1
3	Segesterone Acetate	2.089	352565	39991		1.66	5572	2
4	Ethinyl Estradiol	5.338	3881444	231045	9.92	1.83	5689	2
5	Segesterone Acetate	2.089	357977	40397		1.68	5531	3
6	Ethinyl Estradiol	5.327	3896953	231968	9.91	1.86	5713	3

Assay (sample)

Table 6: Peak Results for Assay sample

S no	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Segesterone Acetate	2.088	352291	40268		1.69	5517	1
2	Ethinyl Estradiol	5.276	3883795	231355	9.75	1.89	5678	1

3	Segesterone Acetate	2.087	356548	41158		1.72	5556	2
4	Ethinyl Estradiol	5.268	3896494	234962	9.82	1.91	5805	2
5	Segesterone Acetate	2.085	358915	40964		1.75	5488	3
6	Ethinyl Estradiol	5.262	3900104	233542	9.78	1.95	5791	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{}{\text{Label claim}} \times 100$$

The % purity of Segesterone Acetate and Ethinyl Estradiol in pharmaceutical dosage form was found to be100.5%.

Linearity
Chromatographic data for linearity study:
Segesterone Acetate

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33.3	5	134437
66.6	10	245572
100	15	371549
133.3	20	499025
166.6	25	619831

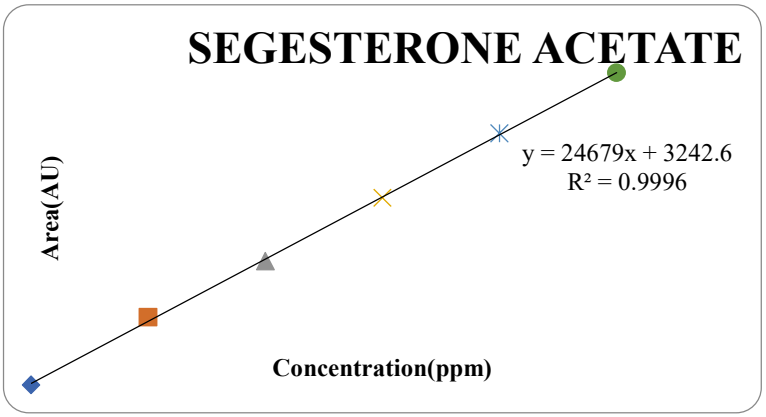

Fig 3: calibration graph for Segesterone Acetate

Ethinyl Estradiol

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	45	1330055
66	90	2728975
100	135	3917064
133	180	5300023
166	225	6412696

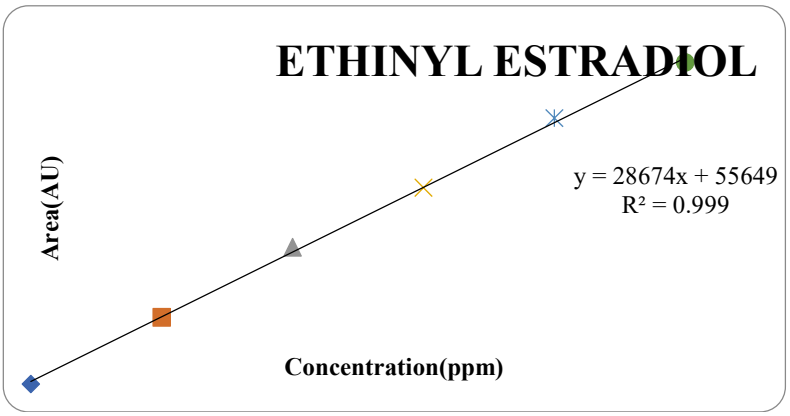

Fig 4: calibration graph for Ethinyl Estradiol

Repeatability

Table 7: Results of repeatability for Segesterone Acetate

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Segesterone Acetate	2.086	362267	41698	5082.3	1.8
2	Segesterone Acetate	2.083	364903	41403	5145.1	1.8
3	Segesterone Acetate	2.083	366871	41541	5119.1	1.8
4	Segesterone Acetate	2.081	367274	42257	5148.3	1.8
5	Segesterone Acetate	2.081	368102	42144	5102.8	1.8
Mean			365883.4			
Std. Dev			2338.314			
% RSD			0.639087			

- *%RSD for sample should be NMT 2*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Table 8: Results of method precession for Ethinyl Estradiol

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Ethinyl Estradiol	5.178	3903549	240180	5989.3	2.1	9.8
2	Ethinyl Estradiol	5.199	3905818	235524	5857.3	2.0	9.7
3	Ethinyl Estradiol	5.235	3916121	238579	5931.2	2.0	9.9
4	Ethinyl Estradiol	5.202	3916543	238815	5937.9	2.0	9.8
5	Ethinyl Estradiol	5.206	3920944	241007	5041.0	2.0	9.5
Mean			3912595				
Std. Dev			7508.046				
% RSD			0.191894				

- *%RSD for sample should be NMT 2*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Intermediate precision
Day 1

Table 9: Results of Intermediate precision for Segesterone Acetate

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Segesterone Acetate	2.083	369247	42278	5538.8	1.6
2	Segesterone Acetate	2.083	370767	42709	5562.8	1.6
3	Segesterone Acetate	2.089	370841	42066	5488.3	1.6
4	Segesterone Acetate	2.083	370842	42067	5490.3	1.6

5	Segesterone Acetate	2.082	371043	42569	5584.2	1.8
6	Segesterone Acetate	2.080	371387	42212	5534.2	1.8
Mean			370687.5			
Std. Dev			740.7368			
% RSD			0.18			

- *%RSD of five different sample solutions should not more than 2*

Table 10: Results of Intermediate precision for Ethinyl Estradiol

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Ethinyl Estradiol	5.229	3743004	242956	5268.7	2.2	10.2
2	Ethinyl Estradiol	5.203	3845358	242254	5101.5	2.1	10.0
3	Ethinyl Estradiol	5.133	3885015	242853	5128.6	2.1	10.0
4	Ethinyl Estradiol	5.229	3743004	242957	5268.7	2.2	10.2
5	Ethinyl Estradiol	5.151	3722514	240345	5049.8	1.5	9.9
6	Ethinyl Estradiol	5.112	3728788	237639	5998.2	1.6	9.9
Mean			3777948				
Std. Dev			69193.4				
% RSD			1.9				

- *%RSD of five different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is rugged.*

Day 2

Table 11: Results of Intermediate precision Day 2 foR Segesterone Acetate

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Segesterone Acetate	2.078	370978	42979	3084.0	1.9
2	Segesterone Acetate	2.082	371042	42569	3584.2	1.8
3	Segesterone Acetate	2.080	371387	42212	3532.2	1.8
4	Segesterone Acetate	2.089	369247	42278	1538.8	1.6
5	Segesterone Acetate	2.083	370841	42066	1488.3	1.6
6	Segesterone Acetate	2.089	369247	42278	1536.8	1.6
Mean			370457.4			
Std. Dev			954.6006			
% RSD			0.27			

- *%RSD of five different sample solutions should not more than 2*

Table 12: Results of Intermediate precision for Ethinyl Estradiol

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Ethinyl Estradiol	5.077	3841405	246819	5209.0	2.1	10.1
2	Ethinyl Estradiol	5.151	3885013	242855	5128.6	2.1	10.0
3	Ethinyl Estradiol	5.112	3743002	242956	5268.7	2.2	10.2
4	Ethinyl Estradiol	5.133	3743007	242954	5268.7	2.2	10.2
5	Ethinyl Estradiol	5.203	3885015	242853	5126.6	2.1	10.0
6	Ethinyl Estradiol	5.133	3743004	242956	5268.7	2.2	10.2
Mean			3806741				
Std. Dev			71613.48				
% RSD			1.9				

- *%RSD of five different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is rugged.*

Accuracy

The accuracy results for Segesterone Acetate

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	192447.6	7.6	7.3	98.7	98.7%
100%	374223	16	13.8	98.67	
150%	555892.3	21.5	22.4	99.2	

The accuracy results for Ethinyl Estradiol

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	2001753	67.6	67.4	99.7	99.7%
100%	3927798	136	134.9	99.8	
150%	5858666	203.5	202.2	99.8	

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Table 13: Results for robustness

Segesterone Acetate

Parameter used for sample analysis	Peak Area	Retention Time	Theotetical plates	Tailing factor
Actual Flow tate of 1.0 mL/min	372127	2.090	5588	1.70
Less Flow rate of 0.9 mL/min	356766	2.736	5433	1.82
MoRe Flow rate of 1.1 mL/min	342357	1.673	5645	1.91
Less organic phase	312435	2.736	5099	1.82
More organic phase	305624	1.673	5124	1.91

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Ethinyl Estradiol

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	3864999	5.289	5699	1.77
Less Flow rate of 0.9 mL/min	3546738	6.746	5547	1.88
MoRe Flow rate of 1.1 mL/min	3857217	4.032	5123	1.91
Less organic phase	3810346	6.746	5035	1.88
More organic phase	3875643	4.032	5613	1.91

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Segesterone Acetate and Ethinyl Estradiol in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Segesterone Acetate and Ethinyl Estradiol was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: TEA Buffer pH 4.5: Acetonitrile (65:15:20) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This

method can be used for the routine determination of Segesterone Acetate and Ethinyl Estradiol in bulk drug and in Pharmaceutical dosage forms.

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