ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR QUANTIFICATION OF EXEMESTANE IN NANOEMULSION BY RP HPLC

Bhupendra G. Prajapati[a]*, Bhavesh B. Prajapati[a] and Sapna M. Rathod[b]

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ABSTRACT

A simple, precise, reproducible and sensitive RP HPLC method was developed and validated for the quantification of exemestane (EXM) in nanoemulsion. The method was developed using Phenomenex C_{18} column (250 mm × 4.6 mm i.d., 5 µm) and acetonitrile: methanol (40:60 V/V) was used as mobile phase. The flow rate was maintained at 1.0 mL min^{-1}. The analyte was monitored at 249 nm. The analyte shows linear response in the range of 0.5–5 µg mL^{-1}. The developed method was validated as per ICH guidelines for accuracy, precision, limit of detection, limit of quantitation and robustness. The data for precision studies revealed that the method is precise, as % RSD is less than 2. The standard addition method was used for the accuracy study and the method showed 100.3 ± 0.47 % recovery for the drug. The proposed method can be successfully applied to the dosage form.

Keywords: Exemestane, RP HPLC, ICH, nanoemulsion

INTRODUCTION

Breast carcinoma has become a major health problem affecting as many as one in eight women[1]. It is recognized that approximately one-third of all breast carcinomas are estrogen dependent and regress after estrogen deprivation[2]. Exemestane (6-methylenandrosta-1,4-diene-3,17-dione) is used in the treatment of breast cancers such as the hormone-receptor-positive breast cancer in women after menopause (Fig. 1).

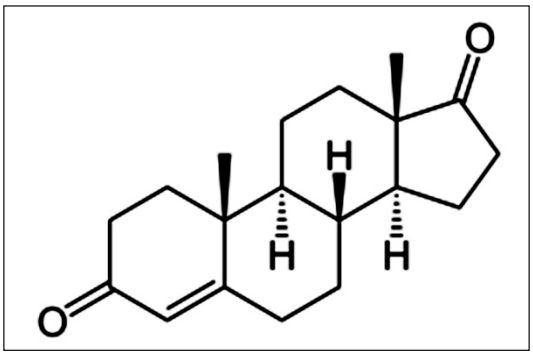

Fig. 1: Structure of exemestane (EXM)

EXM is a steroid analogue, structurally related to the endogenous androstenedione, which irreversibly binds to the active site of the aromatase enzyme involved in the conversion of androgens to estrogens[3]. Plasma estrogen levels are reduced by 85 % to 95 % within 2 to 3 days and effects last 4 to 5 days. Exemestane does not inhibit any of the major cytochrome P450s and has essentially no interaction with steroid receptors, with only a very weak affinity for the androgens receptor (AR). The 17-hydroxyexemestane reduction product, however, has much higher affinity for the AR than the parent (still several fold less than DHT, 0.28 % for parent vs. 30 % for metabolite)[4].

Over the last decades, the development of cancer treatment has been significantly promoted by cancer nanomedicine, which is an interdisciplinary field focusing on the design and medical applications of materials and technologies at the nanoscale (typically up to 100 nm)[5]. Nanoparticulate-based delivery systems provide many potential benefits, including increased biocompatibility, multifunctional encapsulation of active agents and reduced degradation during blood circulation, passive or active targeting, effective delivery and reduced or eliminated side effects[6].

Literature survey reports that exemestane can be determined by different analytical techniques such as HPTLC[7], UV-spectrophotometry[8,9], GC-MS[10], LC-MS[11-15],

[a] Department of Pharmaceutics, Shree S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Mehsana-Gozaria Highway, Kherva - 384 012, Gujarat, India

[b] Department of Pharmaceutical Chemistry and Quality Assurance, APMC College of Pharmaceutical Education and Research, Motipura, Himatnagar – 383 001, Gujarat, India

*For Correspondence: E-mail: bhupen27@gmail.com, bhupendra.prajapati@guni.ac.in

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HPLC[16-18], stability indicating HPLC method[19-21] and UPLC[22] in pharmaceutical dosage forms. Consequently, a simple, precise, reproducible and sensitive RP HPLC method was developed and validated for the quantification of exemestane (EXM) in nanoemulsion.

MATERIALS AND METHODS

Apparatus

A Shimadzu (Columbia, MD) RP-HPLC instrument (LC-2010CHT) equipped with a UV-Visible detector and a photodiode array detector, manual injector with 20 mL loop, Phenomenex (Torrance, CA) C18 column (250 mm × 4.6 mm id, 5 µm particle size) and LC-solution software was used.

Materials

Exemestane was kindly gifted by Astron Research Centre, Ahmedabad, Gujarat (India). Caprol Micro Express was gifted by Abitec Corp., USA. Tween 80, triacetin and PEG 400(A.R.) were obtained from S.D. Fine Chem Ltd., Mumbai, India.

Preparation of standard solution

Accurately weighed EXM 10 mg was transferred to a 100 mL volumetric flask and dissolved and diluted to the mark with methanol to obtain a standard stock solutions having 100 µg mL^{-1} concentration of EXM.

METHOD DEVELOPMENT

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for EXM was obtained with the mobile phase acetonitrile: methanol (40:60 V/V) at a flow rate of 1.0 mL min^{-1} to get better reproducibility and repeatability. Quantification was achieved with UV detector at 249 nm based on peak area.

METHOD VALIDATION

Specificity

The specificity was ensured by recording the chromatogram of blank and the standard solution of EXM.

Linearity

The calibration curves were plotted over the concentration range 0.5 - 5 µg mL^{-1} for EXM. Accurately measured standard working solutions of EXM (0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 and 5 mL) were transferred to a series of 10 mL of volumetric flasks and diluted to the mark with mobile phase. Aliquots (20 µL) of each solution were injected under the operating chromatographic conditions.

Precision

The precision of the instrument was checked by repeatedly injecting ($n = 6$) standard solutions of EXM (2.5 µg mL^{-1}) and peak area, retention time and tailing factor were measured. The intraday and inter day precision study was done by injecting 3 different concentrations of standard solutions of EXM (1, 1.5 and 3 µg mL^{-1}) in triplicate on the same day and on three consecutive days respectively.

Accuracy

Accuracy study was performed by standard addition technique. Known amounts of standard solutions of EXM were added at 50, 100 and 150 % level to prequantified sample solutions of EXM. Accuracy was studied in triplicate.

Limit of detection and limit of quantitation

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations as per International Conference on Harmonization (ICH) guidelines[23].

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

where σ= Standard deviation of the response

S = Slope of calibration curve

System suitability parameters

The % RSD for parameters such as retention time, number of theoretical plates and tailing factor was determined by injecting six replicates of the standard and sample solution of EXM into the chromatographic system.

Robustness

It was done to confirm that the method remains unchanged by the slight variations in the parameters of the developed method. The mobile phase composition, flow rate as well as wavelength were changed to evaluate the robustness of the method. The robustness was performed using 1.5 µg mL^{-1} standard solution of EXM.

Assay of prepared nanoemulsion

The self-emulsifying nanoemulsion was prepared using Caprol micro express, Labrafac as oil phase,

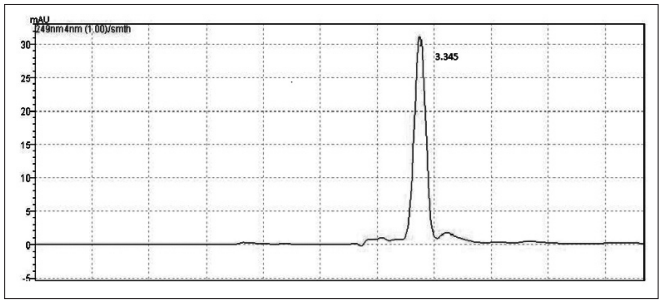

Fig. 2: RP-HPLC chromatogram of standard EXM (0.5 μg mL^{-1})

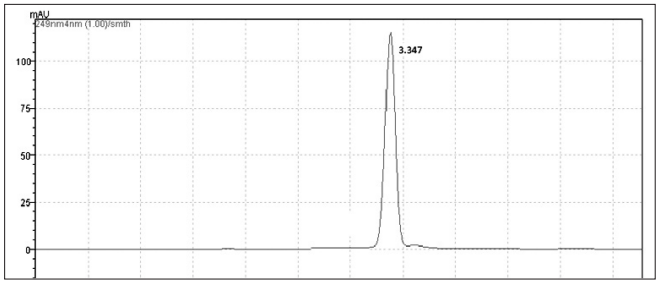

Fig. 3: RP-HPLC chromatogram of standard EXM (5 μg mL^{-1})

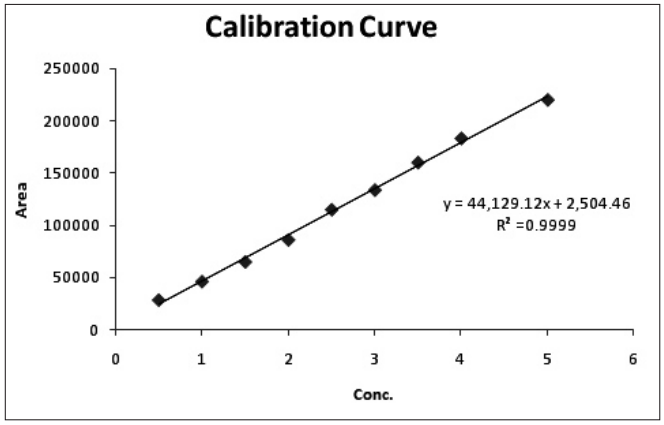

Fig. 4: Calibration curve of EXM

triacetin as cosolvent and Tween 80 as surfactant. 1 mL of nanoemulsion (equivalent to 25 mg exemestane) was taken in a 100 mL volumetric flask and volume was made up to the mark with mobile phase (diluent). Aliquot 1 mL of solution was taken in a 100 mL volumetric flask and the volume made up with diluent. 20 µL of the solution was injected to the chromatographic system. The analysis was carried out in triplicate. The amount of analyte was calculated by substituting the peak area in the regression equation.

RESULTS AND DISCUSSION

Method development

Various combinations of mobile phase were tried for the quantitation of exemestane hydrochloride in nanoemulsion. Finally, the resolved peak was observed using 40:60 % V/V of acetonitrile and methanol at the flow rate of 1 mL min^{-1}. The drug was eluted at 3.345 min. The optimized chromatogram of standard and sample solutions of EXM are shown in Figs. 2 and 3. The specificity of the method was verified by injecting blank solution and no interference of blank was observed at the retention time of EXM. The peak purity of EXM was done to determine there is no co - elution of the other components with the drug.

Table I: Summary of validation parameter of EXM

Parameter	RP-HPLC Method
	EXM
Detection wavelength (nm)	249
Concentration range (µg mL^{-1})	0.5-5
Slope	44129
Intercept	2504
Correlation coefficient	0.997
LOD (µg mL^{-1})	0.08
LOQ (µg mL^{-1})	0.24
Accuracy (n = 6), % recovery ± SD	100.3 ± 0.47 %
Repeatability (n = 6), % RSD	0.34 %
Precision, % RSD	
Interday (n = 6)	1.05 - 1.15 %
Intraday (n = 6)	0.25 - 1.28 %

Table II: Repeatability data for EXM

EXM (1.5 μg mL^{-1})	Retention time (min)	Peak area	Tailing factor
1	3.34	65000	1.01
2	3.32	65102	1.02
3	3.33	65309	1.03
4	3.35	64932	1.01
5	3.34	64821	1.04
6	3.33	65400	1.02
Mean	3.335	65094	1.025
SD	0.010	223.398	0.010
% CV	0.314	0.343	1.023

Linearity

The proposed method follows Beer's law in the concentration range of 0.5 – 50 µg mL^{-1} of EXM with a correlation coefficient of 0.999. The calibration plot for the developed method is represented in Fig. 4. The

Table III: Accuracy data of EXM

Drug	Level	Amount of sample taken (μg mL^{-1})	Amount of standard spiked (%)	Mean % Recovery ± SD
EXM	I	5	50 %	100.3 ± 0.47
	II	5	100 %	100.2 ± 0.65
	III	5	150 %	100.1 ± 0.33

Table IV: Summary of system suitability parameters

Parameter	TEST EXM (2.5 μg mL^{-1}) ± % RSD, n = 6	STD EXM (2.5 μg mL^{-1}) ± % RSD, n = 6
Retention time (min)	3.345 ± 0.07	3.347 ± 0.19
Tailing factor	1.012 ± 0.26	1.121 ± 0.18
Theoretical plates	9397.48 ± 0.53	9098.79 ± 0.27

Table V: Result of robustness study of EXM

Sr. No.	Variation	Parameters	Mean RT ± % RSD (n = 3)	Mean peak area ± % RSD (n = 3)
1	Change in flow rate	0.9 mL min^{-1}	3.359 ± 0.096	115212 ± 1.444
		1.1 mL min^{-1}	3.338 ± 0.118	117813.3 ± 1.654
2	Change in proportion of mobile phase (ACN : MeOH, %V/V)	38 : 62	3.442 ± 0.105	115223.3 ± 1.568
		42 : 58	3.449 ± 0.089	115741.3 ± 1.598
3	Change in Wavelength (± 2 nm)	247	3.345 ± 0.068	117688.3 ± 0.775
		251	3.348 ± 0.035	115133 ± 1.243

regression parameters for the calibration are tabulated in Table I.

Precision

The % RSD value for the repeatability study was found to be 0.34 %. The result of repeatability study is was tabulated in Table II. The replicate analysis was done using three concentration levels for intraday and interday precision study. The % RSD was calculated.

Table VI: Assay results of EXM

Sr. No.	% Assay
1	99.86
2	99.82
3	98.98
4	99.44
5	100.89
6	101.12
Mean	100.02
SD	0.831
% RSD	0.61

The results are represented in Table I and indicate good reproducibility of the method.

Accuracy

The accuracy of the method was determined by adding the standard solution of the drug at three levels (50, 100, and 150 %) to the pre-analysed sample solution of EXM. The study was done three times and the results are shown in Table III. The method showed good mean recovery of 100.3 ± 0.47 %.

Limit of detection and limit of quantitation

The limit of detection and Limit of quantitation for EXM was found 0.08 µg mL^{-1} and 0.24 µg mL^{-1}, respectively. The data shows that the method is sensitive for estimation of EXM in nano emulsion.

System suitability

The % RSD was measured after six replicate injections of the standard as well as sample solution of the EXM under optimized chromatographic condition. The results are shown in Table IV.

Robustness

The results of robustness study verify that the method remains unchanged due to slight changes in the method parameters. The results are shown in Table V.

Assay of prepared nanoemulsion

The developed method was applied to the determination of EXM in nanoemulsion. The results are shown in Table VI and indicate that the method is suitable for the quantitation of EXM in nanoemulsion.

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