Comparison of Two HPLC Methods for the Quantitative Estimation of Exemestane from Protein Nanoparticles-A preliminary study

Samipta Singh[1], Shubhini A. Saraf[2]

[1,2]*Department of Pharmaceutical Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow-226025*

Abstract: *Exemestane is a drug used for breast cancer management. The study aimed to evaluate two different analytical methods for quantitative estimation of Exemestane. One method utilized Acetonitrile and water as a mobile phase (85:15) and flow rate was 1 ml/min. Another method utilized methanol and water as a solvent (95:05) with a 0.5 ml/min flow rate. Both the methods were compared for their accuracy in drug content when they are in a formulation form. Analyses of Exemestane from both methods were in good agreement with the claimed amount of the drug at the label. However, for estimation of protein nanoparticles, Acetonitrile and water method was found to be better.*

Keywords: **Protein nanoparticles, whey protein, HPLC**

1. Introduction:

Exemestane is an aromatase inhibitor recommended to post-menopausal breast cancer women as an adjuvant therapy [8]. Exemestane inhibited human placental aromatase. It caused irreversible time-dependent aromatase inactivation. In 1986, the drug entered preclinical development and received FDA approval in 1999 [5]. Aromatase is an enzyme responsible for estradiol and estrone biosynthesis from androgens. Aromatase inhibition results in estrogen deprivation [2].

In the past, Yavuz et al (2007) reported an HPLC method using Acetonitrile:water 44:56 as mobile phase, stock solutions were prepared in methanol and UV-detection was carried out at 249 nm [9]. Konda et al. (2011) utilized Acetonitrile is to water in a ratio of 60:40. UV detection was carried out at 242 nm [4]. Annapurna et al. (2014) and Breda et al. (1993) utilized phosphate buffer and Acetonitrile (40:60 and 35:65, respectively) as a mobile phase. UV detection was carried out at 247 nm [1, 3]. Another method developed by Mukthinuthalapati and Bukkapatnam (2015) utilized acetate buffer and Acetonitrile (30:70) [6]. Despite extensive work on its analysis, a more rapid and precise HPLC method was desired.

Current research work aims to compare two RP-HPLC methods using two different solvents with respect to their drug content estimation from marketed tablet and nanoparticles. Protein nanoparticles were utilized as model nanoparticles.

2. Materials and methods:

2.1. Materials

Exemestane was gratuitously received from Cipla Pharmaceuticals Limited, Mumbai, Methanol and Acetonitrile were purchased from Merck. Water for HPLC was purchased from SD Fine Chemicals.

2.2. Chromatographic conditions:

Chromatographic separation was executed using a C18 column (5 µm) of the HPLC system (Shimadzu; Model SPD-20A), equipped with a Prominence UV detector.

Corresponding author*: Samipta Singh

2.3. Using methanol: water:

Another isocratic method using methanol as an organic solvent was developed, with methanol and water ratio of 95:05 as a mobile phase. The UV detection was at 242 nm. The stock solution (1 mg/ml) and dilutions (0.1-100 µg/ml) in this method were prepared using methanol as a solvent. Flow rate was 0.5 ml/min.

2.4. Using Acetonitrile: water:

The isocratic elution was carried out using water: Acetonitrile (15:85). Konda et al utilized the same solvents with different mobile phase ratio (40:60) [4]. The flow rate was 1 ml/min. 20 µL was injected into the HPLC system. UV detection was done at 242 nm. Stock solution of 1 mg/ml of Exemestane diluted with Acetonitrile (1000 µg/ml) and further dilutions (1-100 µg/ml) were made with Acetonitrile. Different concentrations of Exemestane were prepared and were run into the HPLC to record the area under the peak for each concentration of Exemestane. Calibration curve was constructed by plotting exemestane concentration vs. area under the curve ratio (Figure 1).

2.5. Marketed nanoparticles

Xtane® was crushed and dissolved in respective solvent, filtered, diluted and estimated for exemestane content using both methods of HPLC.

2.6. Nanoparticle preparation and characterization:

The nanoparticles were by desolvation method, as carried out by Teng et al (2012) with some modifications [7]. The whey protein was dissolved in water; 5 mg exemestane dissolved in ethanol was added to it, followed by the addition of 1% genipin. They were diluted using same ratio of ethanol and water for particle size using zetasizer (Nanoplus 3). For drug content estimation, the nanoparticles were dissolved in 1% SDS and 0.8 mM urea, diluted with HPLC grade acetonitrile/methanol and water and were subjected to exemestane estimation by HPLC.

3. Results and discussion:

3.1. UltraViolet-visible spectrophotometric detection:

Upon UV-detection using a UV-visible spectrophotometer, we found methanol to be the better solvent for UV detection of Exemestane because its λmax did not vary upon changing the concentration because of better solubility of Exemestane in methanol. λmax was found to be 242 nm. The λmax reported by Konda et al. was also 242 nm.

3.2. Methanol: water method:

The method with 95:05 (methanol: water) and 0.5 ml/ min flow rate had good symmetry, area, height and least deviation in retention time upon changing the concentration from 100 µg/ml (7.623 min) to 10 µg/ml (7.625 min). The chromatogram and calibration plot are illustrated in Figures 1 and 2, respectively.

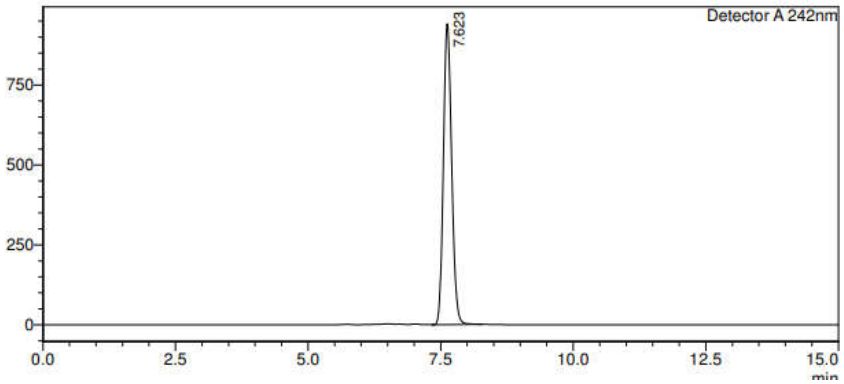

Figure 1: Chromatogram of Exemestane using methanol: water (95:05) as a mobile phase

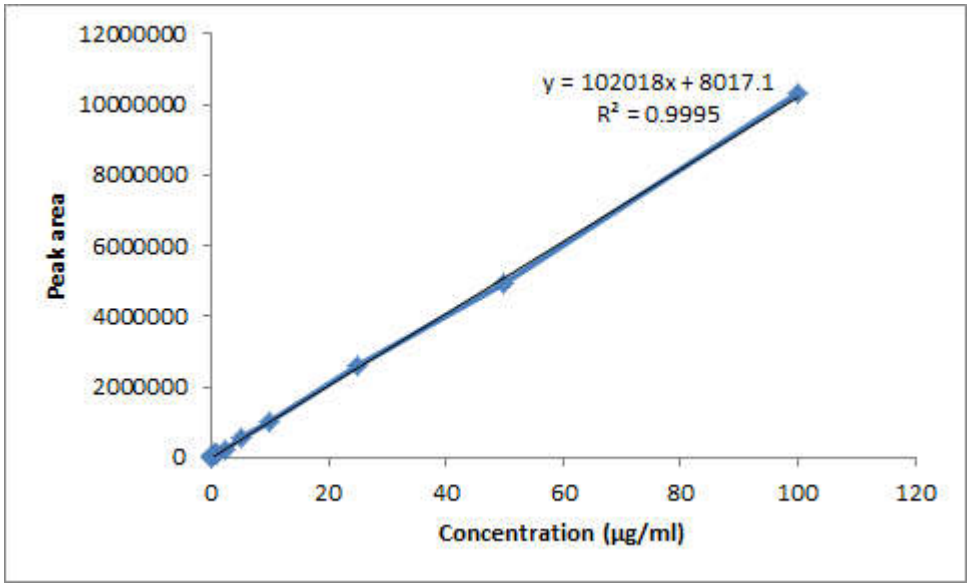

Figure 2: Calibration plot of Exemestane (using mobile phase methanol: water 95:05)

3.3. Acetonitrile: water method:

The peak obtained 85:15 (Acetonitrile: water) also had good symmetry, area and height. The retention time remained constant upon changing the concentration (i.e. 4.7 minutes). This indicated that retention time can be reduced without having its symmetry affected. Moreover, upon varying the λmax and solvent of stock solution, the retention time remained constant. The chromatogram and calibration plot are illustrated in figure 3 and 4 respectively.

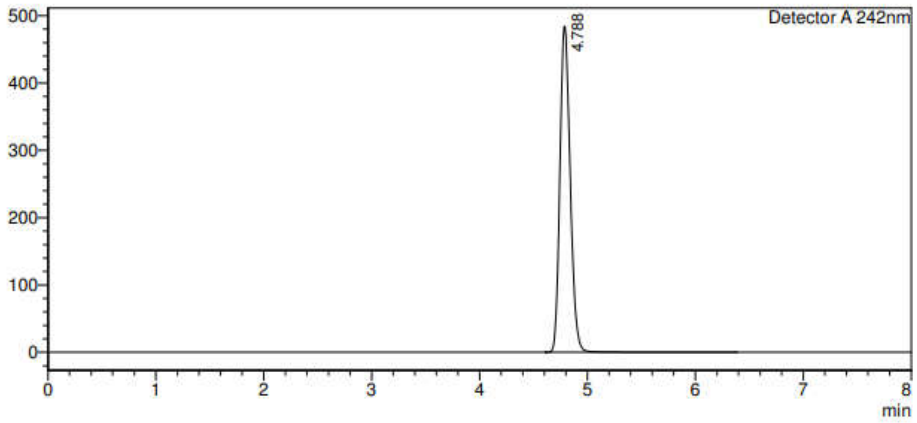

Figure 3: Chromatogram of Exemestane using Acetonitrile: water (85:15) as a mobile phase

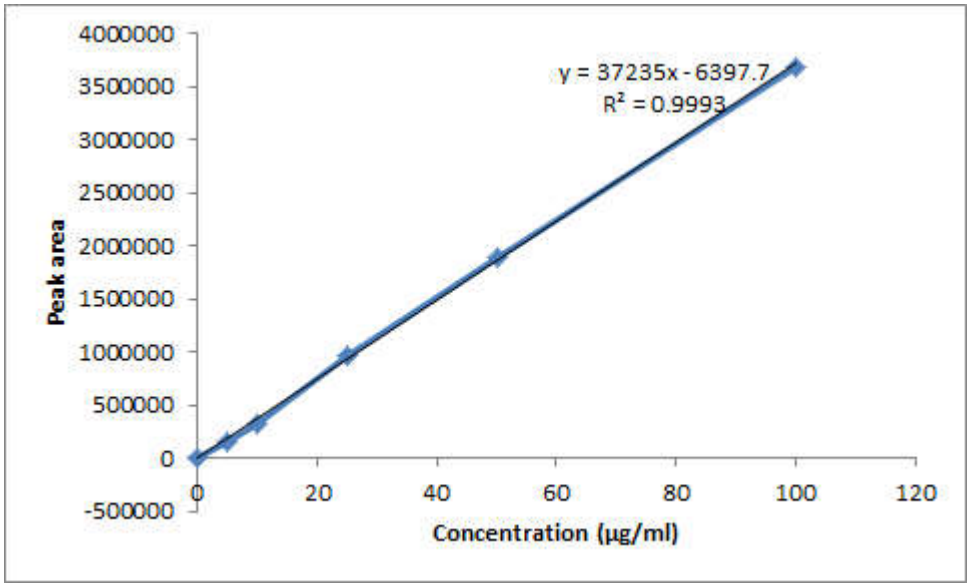

Figure 4: Calibration plot (Acetonitrile: water 85:15)

3.4. Estimation of drug content from formulations:

Protein nanoparticles were used as model nanoparticles to study the effect of other substances on the peak of Exemestane. The size obtained by zetasizer was < 500 nm (illustrated in figure 5). It could be concluded that although methanol gave good results for the marketed formulation was analyzed, Acetonitrile and water gave the results nearer to accurate for protein nanoparticles. Thus, the Acetonitrile: water method was better than the methanol: water method for estimating Exemestane from protein nanoparticles.

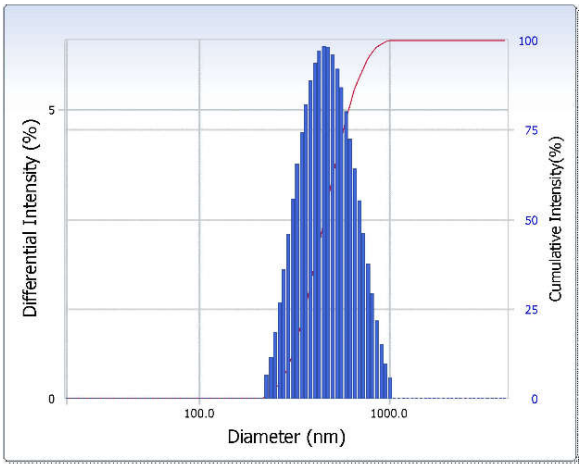

Figure 5: Intensity distribution of protein nanoparticles, as obtained by Nanoplus 3

Table 1. % Drug Content from Various Formulations

Formulation	Method	Drug content (%)
Marketed Exemestane (Xtane®)	Methanol: water	99.99
	Acetonitrile:water	99.98
Formulated Nanoparticles	Methanol: water	126.1
	Acetonitrile: water	99.97

4. Conclusion:

The Acetonitrile: water method was better for the formulation estimation of protein nanoparticles.

5. Acknowledgements:

Priyanka Maurya and Priya Singh (Research Scholars at Department of Pharmaceutical Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow)

6. Reference:

1 M. M. Annapurna, B. Swati, D. N. Ramya, K. Pramadvara, A. Ram, and N. Aslesha, "A Validated Stability-Indicating Liquid Chromatographic Method for the Determination of Exemestane", *Chemical Science,* 3 (2014), pp. 961-68.

2 C. Avendaño, and J. C. Menéndez, "Chapter 3 - Anticancer Drugs That Modulate Hormone Action', in *Medicinal Chemistry of Anticancer Drugs (Second Edition)"*, edited. by C. Avendaño and J. C. Menéndez, Boston: Elsevier, (2015), pp. 81-131.

3 M. Breda, E. Pianezzola, and M. S. Benedetti, "Determination of Exemestane, a New Aromatase Inhibitor, in Plasma by High-Performance Liquid Chromatography with Ultraviolet Detection", *Journal of Chromatography B: Biomedical Sciences and Applications,* 620, (1993), pp. 225-31.

4 B. Konda, R. N. Tiwari, and H. Fegade, "Development and Validation of Stability Indicating Method for the Determination of Exemestane by Reverse Phase High Performance Liquid Chromatography", *Journal of chromatographic science,* 49, (2011), pp. 634-39.

5 P. Lombardi, "Exemestane, a New Steroidal Aromatase Inhibitor of Clinical Relevance", *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease,* 1587, (2002), pp. 326-37.

6 M. A. Mukthinuthalapati, and V. Bukkapatnam, "A Novel Validated Stability-Indicating Rp-Hplc Method for the Determination of Exemestane (Steroidal Aromatase Inhibitor)", *Journal of Bioequivalence & Bioavailability,* 7, (2015), pp. 288.

7 Z. Teng, Y. Luo, and Q. Wang, "Nanoparticles Synthesized from Soy Protein: Preparation, Characterization, and Application for Nutraceutical Encapsulation", *Journal of Agricultural and Food Chemistry,* 60, (2012), pp. 2712-20.

8 Y. Wang, F. Jing, and H. Wang, "Role of Exemestane in the Treatment of Estrogen-Receptor-Positive Breast Cancer: A Narrative Review of Recent Evidence", *Advances in Therapy,* 39, (2022), pp. 862-91.

9 B. Yavuz, E. Bilensoy, and M. Şumnu, "Analytical Method Validation for HPLC Assay of Oral Anticancer Drug Exemestane", *FABAD J. Pharm. Sci.,* 32, (2007), pp. 15-22.