METHOD DEVELOPMENT AND VALIDATION FOR THE GC-FID ASSAY OF 17 β-ESTRADIOL IN PHARMACEUTICAL PREPARATION

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ABSTRACT

A simple and rapid method for determination of 17 β-estradiol in pharmaceutical preparation was developed and validated using gas chromatography with flame ionization detection (GC-FID). The solutions of standard and the sample were prepared in methanol. GC separation was performed in about 7.7 min using a 30 m x 0.32 mm I.D. (film thickness 0.25 µm) HP-5 capilary column. Nitrogen was used as carrier gas at a flow-rate of 2 ml min^{-1}. After injection of the sample at inlet temperature 250 ^{0}C, the temperature of the GC oven was as follows: initial temperature was 150 ^{0}C, held for 1.5 min, increased to 260 ^{0}C at a rate of 50 ^{0}C min^{-1} held for 5 min, and finally to 270 ^{0}C at a rate of 10 ^{0}C min^{-1} and held for 3.3 min. Detector temperature is 300 ^{0}C. 2 µl was injected in splitless mode. Calibration curves were linear between the concentration range 0.25-50 µg ml^{-1}. The method was validated for specificity, linearity, precision, accuracy and limit of quantitation. Also, the method was applied to directly and easily to the analysis of the pharmaceutical preparation (Estrofem tablet).

Keywords: 17 β-estradiol, GC-FID, Pharmaceutical preparation

INTRODUCTION

17 β-estradiol (Figure 1) is the most potent of the natural human estrogens [1]. 17 β-estradiol, chemically 1, 3, 5 (10)-estratrien-3, 17 β-diol, is the most potent estrogen of a group of endogenous estrogen steroids which includes estrone and estriol. It is responsible for the growth of breast and reproductive epithelia, maturation of long bones, and development of secondary sexual characteristics.

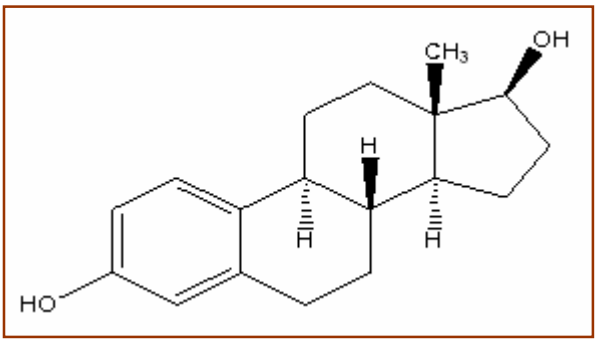

Figure 1: Chemical structure of 17 β-estradiol

17 β-estradiol and its semi-synthetic esters are primarily used as menopausal hormones. It may also be used as replacement therapy for female hypogonadism or primary ovarian failure. The decrease of 17 β-estradiol at menopause is often accompanied by vascular instability and rise in incidence of heart disease and an increasing risk of osteoporosis [2].

Several methods have been reported for determination of 17 β-estradiol including voltametric [3], high performance liquid chromatography [4-9]. Many other gas chromatography-mass spectrophotometry (GC-MS) methods have been published quantifying 17 β-estradiol and its metabolites [10–15].

No previous GC-FID method for the determination and quantification of 17 β-estradiol in pharmaceutical preparations in literature. Therefore, the purpose of this investigation was to develop and validate a method using a simple, rapid, sensitive, precise, accurate and specific GC-FID method.

MATERIALS AND METHODS

Chemicals and reagents

17 β-estradiol was purchased from Sigma (St. Louis, Mo, USA), methonol (HPLC grade) from Fluka (Buchs, Switzerland), and other chemicals and solvents used were of analytical grade. Estrofem tablet containing 2 mg of 17 β-estradiol was obtained by pharmacy (Erzurum, Turkey).

Instrumentation

The GC-FID system was performed an Agilent 6890 N Network GC equipped with a flame ionization detector, Agilent 7683 series autosampler, Agilent chemstation and HP-5 column with 0.25 µm film thickness (30 m x 0.320 mm I.D.). Injection and detector temperature is 250 and 300 ^{0}C, respectively. 2 µl was injected in splitless mode. The carry gas (N_2) flow-rate was kept constant during the run at 2 ml min^{-1}. Nitrogen (30 ml min^{-1}), hydrogen (44 ml min^{-1}) and synthetic air (400 ml min^{-1}) were used as auxiliary gases for the flame ionization detector.

Preparations of the standard and quality control solutions

The stock standard solution of 17 β-estradiol was prepared in methanol to a concentration of 100 µg ml^{-1} and stored at -20 ^{0}C. Working standard solutions were prepared from the stock solutions. A calibration graph was constructed in the range of 0.25, 0.5, 1, 3, 5, 10, 12.5, 15, 20, 30, 40 and 50 µg ml^{-1} for 17 β-estradiol (n=6). For quality control (QC) samples containing concentration 1.25, 7.5, 25 µg ml^{-1} of 17 β-estradiol, the stock solution was diluted with methanol.

RESULTS and DISCUSSION

Method development and optimization

The method development for the assay of 17 β-estradiol was based on its chemical properties. 17 β-estradiol is a polar molecule and, therefore, a polar solvent methanol was used as the diluent. The capillary column coated with 5% phenyl, 95% dimethylpolysiloxane is a good choice for separation of this analyte since they elute as symmetrical peaks at a wide range of concentrations.

The GC-FID parameters used in the method development were based on the boiling point. The injection port and detector temperature were set to 250 and 300^0C, respectively. Different temperature programs were investigated for GC oven. The end of this investigation, the best temperature program was selected for a good resolution. The temperature programs of the GC oven with a run time of 13 min was as follows: initial temperature 150^0C, held for 1.5 min, increased to 260^0C at a rate of 50^0C min^{-1} held for 5 min, and finally to 270^0C at a rate of 10^0C min^{-1} and held for 3.3 min. The head pressure was set to ensure a hydrogen flow of 44 ml min^{-1}. The splitless mode was chosen. The solvent, column and acquisition parameters were chosen to be a starting point for the method development.

The retention time of 17 β-estradiol was approximately 7.7 min with good peak shape. No further optimisation of the method was required. Additionally, preliminary precision and linearity studies performed during the development of the method showed that the 2 µl injection volume was reproducible and the peak response was significant at the analytical concentration chosen. Typical chromatogram obtained with standard 17 β-estradiol is presented in Figure 2.

Method validation

Linearity

The linearity of peak area response versus concentration for 17 β-estradiol was studied between concentration range of 0.25-50 µg ml^{-1}. The calibration curve constructed was evaluated by its correlation coefficient. The calibration equation from six replicate experiments, y = 2.0679x-0.9516 (r = 0.9972), demonstrated the linearity of the method. Standard deviations of the slope and intercept for the calibration curves were 0.0179 and 0.0191, respectively.

Precision and accuracy

The precision of the analytic method was determined by repeatability (within-day) and intermediate precision (between-day). Three different concentrations which were QC samples (1.25, 7.5, 25 µg ml^{-1}) were analyzed six time in one day for within-day precision and once daily for three days for between-day precision.

The RSD values for within-day precision was ≤3.02% and for between-day precision was ≤3.82% . The bias values for within-day accuracy was ≤4.56% and for between-day accuracy was ≤5.12%. These values are summarised in Table 1.

Recovery

To determine the accuracy of the proposed method and to study the interference of formulation additives, the recovery was checked as three different concentration levels (5, 10, 25 µg ml^{-1}) and analytical recovery experiments were performed by adding known amount of pure drugs to pre-analyzed samples of commercial dosage form. The percent analytical recovery values were calculated by comparing concentration obtained from the spiked samples with actual added concentrations. These values are also listed in Table 2.

Table 1: Precision and accuracy of 17 β-estradiol by GC-FID method

Added (µg ml^{-1})	Within-day			Between-day		
	Found±SD (µg ml^{-1})	Accuracy	Precision RSD%[a]	Found±SD (µg ml^{-1})	Accuracy	Precision RSD%[a]
1.25	1.29±0.039	3.20	3.02	1.31±0.050	4.80	3.82
7.5	7.61±0.136	1.47	1.79	7.72±0.172	2.93	2.23
25	26.14±0.397	4.56	1.52	26.28±0.752	5.12	2.86

SD: Standard deviation of six replicate determinations, RSD: Relative standard deviation,
[a]Average of six replicate determinations, Accuracy: (%relative error) (found-added)/addedx100

Table 2: Recovery values of 17 β-estradiol pharmaceutical preparation

Commercial preparation	Estrofem tablet (5 µg ml^{-1})			
Method	Added (µg ml^{-1})	Found±SD (µg ml^{-1})	Recovery (%)	RSD[a] (%)
GC-FID	5	4.95±0.192	99.0	3.88
	10	10.14±0.263	101.4	2.59
	25	25.53±0.631	102.1	2.47

SD: Standard deviation of six replicate determinations, RSD: Relative standard derivation
[a]Average of six replicate determinations

Table 3: Stability of 17 β-estradiol in solution

Stability (%)	Room temperature stability (Recovery % ± SD)		Refrigeratory stability, +4 °C (Recovery % ± SD)		Frozen stability, - 20 °C (Recovery % ± SD)	
Added (μg ml^{-1})	24 h	72 h	24 h	72 h	24 h	72 h
2	98.2±3.76	97.9±4.18	98.7±4.25	98.4±4.97	98.2±3.47	97.5±3.93
20	101.9±2.85	101.2±3.98	102.3±3.59	103.1±4.42	101.4±2.56	102.4±4.23

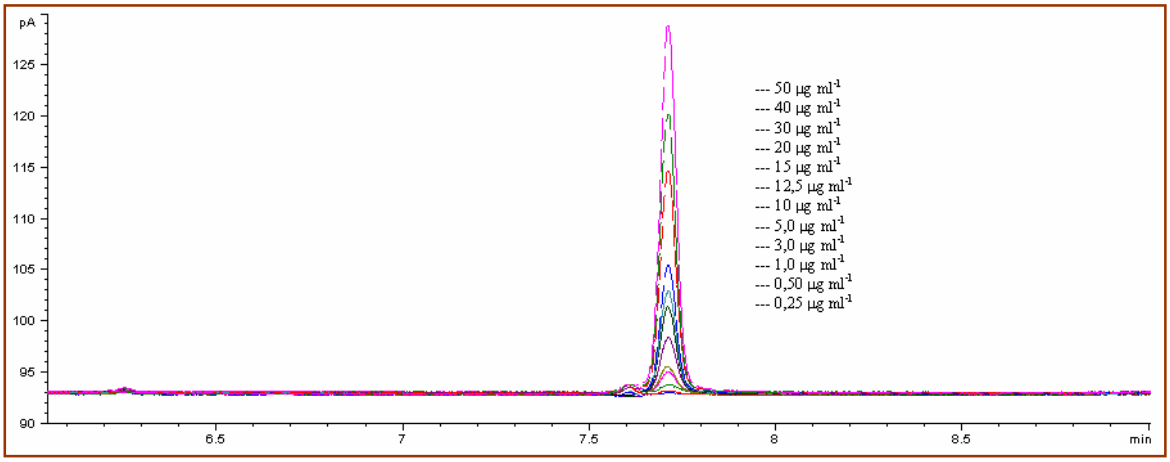

Figure 2: GC-FID chromatograms of 17 β-estradiol

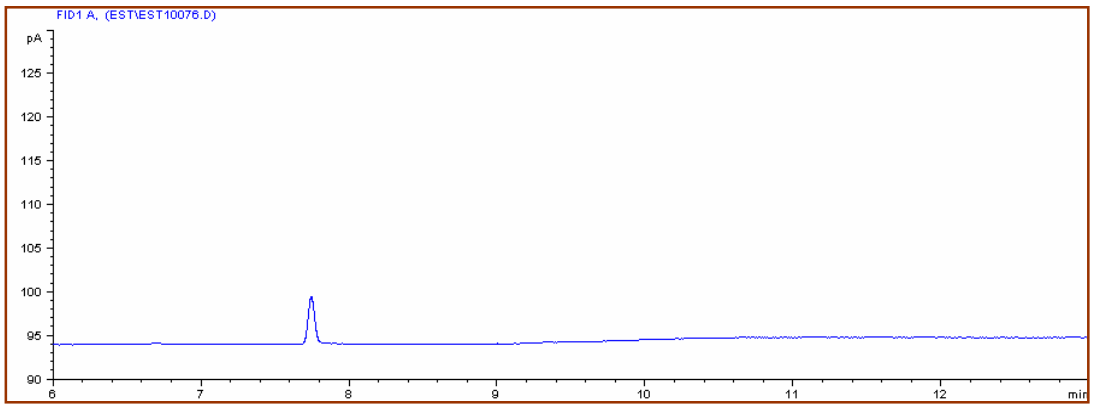

Figure 3: GC-FID chromatogram of Estrofem tablet solution (20 μg ml^{-1})

Limit of detection and quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were evaluated by serial dilutions of 17 β-estradiol stock solutions in order to obtain signal to noise ratios of 3:1 for LOD and 10:1 for LOQ. The LOD and LOQ values for analyte were found to be 0.05 and 0.10 μg ml^{-1}, respectively.

Stability

Stability studies indicated that the samples were stable when kept at room temperature, 4 ^{0}C and -20 ^{0}C refrigeration temperature for 24 h (short-term) and refrigerated at 4 and -20 ^{0}C for 72 h (long-term). The results of these stability studies are given in Table 3, where the percent ratios are within the acceptance range of 90-110%.

Assay sample preparation

Ten tablets of Estrofem (contained 2 mg 17 β-estradiol per tablet) were accurately weighed and finely powdered. An amount of powdered tablet equivalent to about 2 mg of 17 β-estradiol was accurately weighed and dissolved with methanol under ultrasonication for about 15 min at room temperature. Then, obtained solution was filtered with 0.45 mm nylon 25 mm filter and was diluted with methanol to achieve an appropriate an appropriate concentration (20 μg ml^{-1}) (Figure 3).

CONCLUSION

In the present report, a simple, rapid, sensitive, reliable, specific, accurate and precise GC-FID method for the determination of 17 β-estradiol in pharmaceutical preparation was developed and validated. The method

described in the present report has been effectively and efficiently used to analyze 17 β-estradiol pharmaceutical dosage form without any interference from the pharmaceutical excipients. Therefore, GC-FID method can be used for the routine QC analysis of 17 β-estradiol in pharmaceutical preparations.

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