ANALYTICAL SCIENCES MARCH 2010, VOL. 26

Notes

Simultaneous Determination of Estradiol Valerate and Medroxyprogesterone Acetate in a Tablet Formulation by Gas Chromatography–Mass Spectrometry

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A new and simple gas chromatography–mass spectrometry (GC-MS) method has been developed for simultaneous determination of estradiol valerate (EV) and medroxyprogesterone acetate (MPA) in a tablet formulation. The validation of the proposed method was carried out for selectivity, linearity, accuracy, precision, recovery, limits of detection and quantification. The developed method can be used for routine quality control (QC) analysis of titled drugs in combination in tablet formulation.

(Received August 22, 2009; Accepted December 12, 2009; Published March 10, 2010)

Estradiol valerate (EV), 1,3,5(10)-estratriene-3,17β-diol 17-pentanote, is used to treat menopause syndrome and prostate cancer, and can be used together with progestogen for the inhibition of ovulation.[1] Medroxyprogesterone acetate (MPA), 17α-hydroxy-6α-methyl-4-pregnene-3,20-dione 17-acetate, is a synthetic progestational agent used for contraception and treatment of hormone-dependent cancers, especially breast cancer.[2,3] The combination estrogen-gestagen is used for the treatment of estrogenic deficiency syndrome to control its symptoms (climateric syndrome), such as loss of bone minerals and development of heart disease.[4] Up to now, various analytical methods for the determination of EV or MPA have been reported including voltammetry,[1] spectrophotometry,[5,6] fluorometry,[7] high performance liquid chromatography (HPLC),[4,8-11] gas chromatography–mass spectrometry (GC-MS),[12-14] FT-Raman spectroscopy[15] and chemiluminescence enzyme immunoassay[16] in pharmaceutical preparations and biological liquids. But there has been no GC-MS method reported in the litearature for simultaneous determination of these two hormones in pharmaceutical preparations. The analytical methods for their separation and simultaneous quantification are required for QC purposes.

Therefore, the development of a GC-MS method for the determination of EV and MPA in the same tablet dosage form without the necessity of sample pretreatment is required. The proposed method is accurate, sensitive, precise and reproducible and can be directly and easily applied to Divina tablets as pharmaceutical preparation.

Experimental

Chemicals

EV, MPA and 17β-estradiol (internal standard, IS) were obtained from Sigma-Aldrich (St. Louis, MO). Divina tablets containing 2 mg EV and 10 mg MPA were purchased at a pharmacy (Erzurum, Turkey).

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Apparatus and GC/MS conditions

Chromatographic analysis was carried out on an Agilent 6890N gas chromatography system equipped with a 5973 series mass selective detector and a 7673 series autosampler and Chemstation (Agilent Technologies, Palo Alto, CA). HP-5 MS column with 0.25 μm film thickness (30 m × 0.25 mm, USA) was used for separation. Splitless injection was used and the carrier gas was helium at a flow rate of 2 mL/min. The injector and detector temperatures were 250°C. The MS detector parameters were transfer line temperature 280°C, solvent delay 3 min and electron energy 70 eV. The oven temperature program was held at 150°C for 1.5 min, increased to 260°C at a rate of 50°C/min for 1 min and then increased to 270°C at a rate of 10°C/min for 3.3 min.

Preparation of stock and standard solutions

Stock solutions of EV and MPA were prepared by dissolving the accurately weighed reference compounds in methanol to give a final concentration of 100 μg/mL of both. The solutions were then serially diluted with methanol to achieve standard working solutions at concentrations of 100, 200, 500, 1000, 1500, 2000, 2500 ng/mL and 200, 500, 750, 1500, 2000, 2500, 3000 ng/mL for EV and MPA, respectively. A stock solution of IS was prepared in methanol at the concentration of 50 μg/mL and diluted to 5 μg/mL with methanol. All the solutions were stored at 4°C and were brought to room temperature before use. The QC solutions were prepared by adding aliquots of standard working solution of EV and MPA to final concentrations of 300, 1250 and 2250 ng/mL containing 0.1 mL IS (500 ng/mL).

Sample preparation

Ten Divina tablets were weighed and finely powdered. The average weight of tablets was determined with the help of the weight of 10 tablets. A portion of powder equivalent to the weight of one tablet was accurately weighed into a 100-mL volumetric flask and 70 mL methanol was added. The volumetric flask was sonicated for 15 min to effect complete dissolution of the EV and MPA; the solution was then made up to volume with methanol. The solution was filtered through a piece of Whatman No. 42 paper. The aliquot portion of the

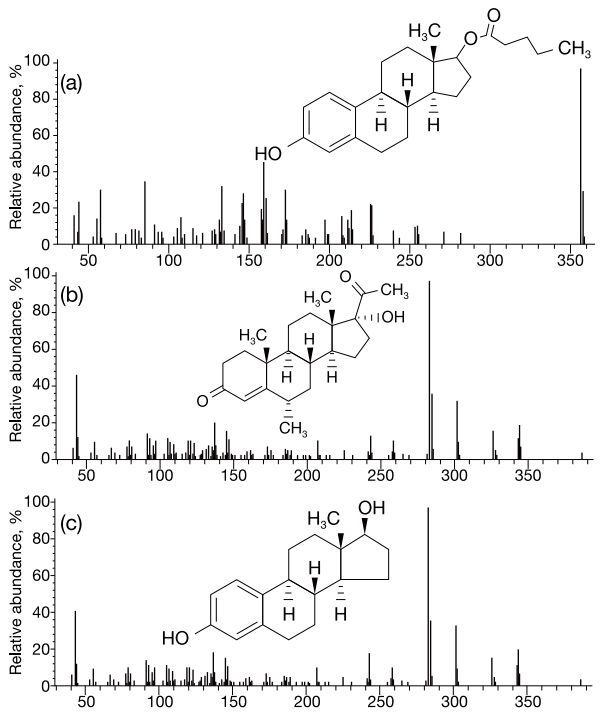

Fig. 1 Structural formula and MS spectra of EV (a), MPA (b) and IS (c).

filtrate was further diluted to get final concentration of 600 ng/mL of EV and 3000 ng/mL of MPA. One microliter of the test solution was injected and the chromatogram was recorded for the same; finally the amounts of the drug were calculated.

Results and Discussion

Validation of the method

The selectivity of the GC-MS method was investigated by observing interferences between EV, MPA and the excipients. For GC/MS, electron impact mode with selected ion monitoring (SIM) was used for quantitative analysis (*m/z* 356 for EV, *m/z* 283 for MPA and *m/z* 272 for IS). The mass spectra of the EV, MPA and IS are shown in Fig. 1. The retention times of EV and MPA in GC-MS method were approximately 10.3 and 11.3 min with good peak shape (Figs. 2a and 2b).

Linearity was determined for EV in the range of 100 – 2500 ng/mL and for MPA in the range of 200 – 3000 ng/mL. The calibration curves were established by plotting the ratio of the peak areas of IS, EV and MPA. The correlation coefficient (*r*) values for both the drugs were >0.99. The regression equations were calculated from the calibration graphs, along with the standard deviations of the slope and intercept on the ordinate. The results are shown in Table 1.

The precision of the GC-MS method was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was evaluated by analyzing QC samples six times per day, at three different concentrations which were QC samples. The intermediate precision was evaluated by analyzing the same samples once daily for three days. The RSD of the predicted concentrations from the regression equation was taken as precision. The accuracy of this analytical method was assessed as the percentage relative error. For all the concentrations studied, intra- and inter-day RSD values were ≤5.44% and for all concentrations of EV and MPA the relative errors were ≤2.47%.

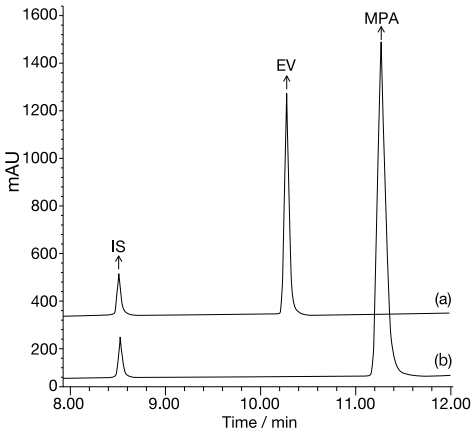

Fig. 2 GC-MS chromatograms of 1000 ng/mL EV and 500 ng/mL IS (a), 2500 ng/mL MPA and 500 ng/mL IS (b).

Table 1 Features of the calibration curves of EV and MPA by GC-MS method

Parameter	EV	MPA
Linearity (ng/mL)	100 – 2500	200 – 3000
Regression equation[a]	$y = 0.0022x + 0.162$	$y = 0.0023x + 0.0566$
Standard deviation of slope	5.2×10^{-4}	5.8×10^{-4}
Standard deviation of intercept	3.2×10^{-4}	3.3×10^{-4}
Correlation coefficient	0.9974	0.9967
Standard deviation of correlation coefficent	5.0×10^{-4}	2.9×10^{-4}
LOD (ng/mL)	15	25
LOQ (ng/mL)	45	75

Based on three calibration curves.
a. *y*, Peak-area ratio; *x*, EV and MPA concentration (ng/mL).

The limit of detection (LOD) and limit of quantification (LOQ) of the method were determined by injecting progressively lower concentrations of the standard solution under the chromatographic conditions. The lowest concentrations were assayed where the signal/noise ratio was at least 10:1, and this concentration was regarded as LOQ. The LOD was defined as a signal/noise ratio of 3:1. The results are shown in Table 1.

To evaluate the stability of EV and MPA, standard solutions were prepared separately at concentrations covering the low, medium and higher ranges of the calibration curve for different temperature and times. These solutions were stored at room temperature, refrigeratory (4°C) temperature or frozen (–20°C) temperature for 6 and 72 h and no significant degradation was observed. These results are within the acceptance range of 90 – 110%.

To determine the accuracy of the GC/MS method and to study the interference of formulation additives, we checked the recovery at three different concentration levels. The analytical recovery experiments were performed by adding known amounts of pure drugs to pre-analyzed samples of commercial dosage form (Divina tablet containing 2 mg EV and 10 mg MPA). The percent analytical recovery values were calculated by comparing concentrations obtained from the spiked samples with actual

Table 2 Recovery of EV and MPA in Divina tablet containing 2 mg EV and 10 mg MPA

Pharmaceutical preparation	Added/ng ml^{-1}	Intra-day		Inter-day	
		Found ± SD[a]	%Recovery (%RSD[b])	Found ± SD[a]	%Recovery (%RSD[b])
EV Divina (2 mg)	300	294.5 ± 9.61	98.2 (3.26)	295.7 ± 11.59	98.6 (3.92)
	1300	1283.1 ± 36.31	98.7 (2.83)	1290.9 ± 48.28	99.3 (3.74)
	2300	2306.7 ± 80.04	100.3 (3.47)	2279.3 ± 96.64	99.1 (4.24)
MPA Divina (10 mg)	250	244.8 ± 5.56	97.9 (2.27)	244.5 ± 9.07	97.8 (3.71)
	1500	1473.0 ± 49.19	98.2 (3.34)	1480.5 ± 74.62	98.7 (5.04)
	2500	2530.0 ± 70.33	101.2 (2.78)	2485.0 ± 97.91	99.4 (3.94)

a. SD, Standard deviation of six replicate determinations. b. RSD, Relative standard deviation.

Table 3 Application of GC-MS method for the determination of EV and MPA in Divina tablet

n	Divina tablet	Found ± SD[a]	%Recovery	%RSD[b]
10	EV (2 mg)	2.03 ± 0.067	101.5	3.3
	MPA (10 mg)	10.12 ± 0.228	101.2	2.25

n, Number of determinations.
a. SD, Standard deviation.
b. RSD, Relative standard deviation.

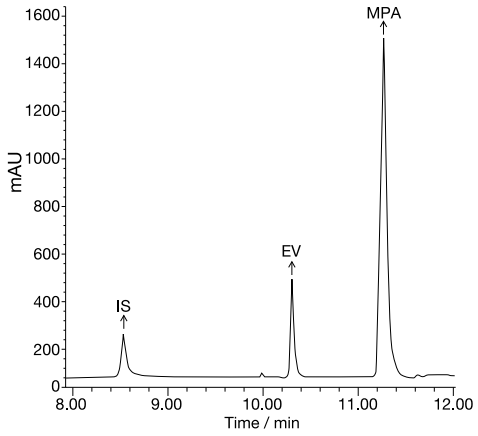

Fig. 3 A typical chromatogram of Divina tablet sample solution containing 600 ng/mL of EV and 3000 ng/mL of MPA.

added concentrations. These values are also listed in Table 2.

Today, HPLC and GC methods are important and widely used as analytical techniques of quantitative and qualitative analysis. As compared to HPLC, high resolution capillary GC has inherently high resolving power and high sensitivity with excellent precision and accuracy. Also, the detection limits were lowered to pg levels by GC combined with MS.[17]

The present work describes the validation parameters stated either by USP 26[18] or by the ICH guidelines[19] to achieve GC/MS method for simultaneous determination of EV and MPA. Also, the developed method was applied to the determination of EV and MPA in the same tablet (Table 3).

Conclusions

In this research, a sensitive and accurate GC-MS method has been developed and validated for quantitative determination of EV and MPA in a tablet formulation. The method is very simple and specific, as both peaks are well separated from its impurities and excipient peaks. Therefore, the proposed method can be used for the routine QC analysis of simultaneous determination of EV and MPA pharmaceutical preparations in a total time of 12 min (Fig. 3).

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