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Contributed Paper

High Performance Liquid Chromatography Incorporating to Short Column and Micellar Mobile Phase for Determination of Some Contraceptive Drugs

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ABSTRACT

In this research, reverse-phase high performance liquid chromatography using green mobile phase utilizing short column was developed for the determination of some contraceptive drugs such as ethinyl estradiol (EE), cyproterone acetate (CPA), gestodene (GES) and levonorgestrel (LNG). This Zorbax SB-C18 (12.5×4.6 mm i.d., 5 µm) that commonly used as guard column has been used instead of the expensive and conventional analytical column. The chromatographic behaviors were studied to confirm the performance of this short column and it was found that the reciprocal value of capacity factor of each analyte was linear to micellar concentration. The optimum conditions were 0.05 mol L^{-1} sodium dodecyl sulfate (SDS) and isopropanol (95:5, v/v) as micellar mobile phase with a flow rate of 1.0 mL min^{-1}. The detection wavelengths were 281, 281, 240 and 240 nm for EE, CPA, GES and LNG, respectively. A green extractant was used in this method instead of organic solvent to extract the analytes. The calibration curves of each contraceptive drug were linear with $R^2 > 0.9990$ and detection limits were 0.003, 0.06, 0.025 and 0.05 µg mL^{-1} for EE, CPA, GES and LNG, respectively. The proposed method was applied successfully for the determination of contraceptive drugs in oral contraceptive pills. The developed system is not only a green analytical approach but also an effective and inexpensive method for this type of analysis.

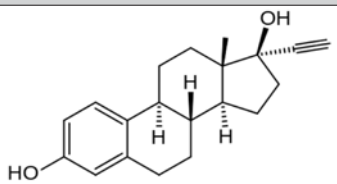
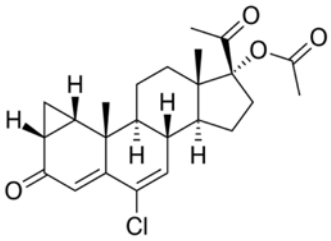
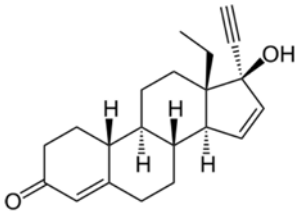
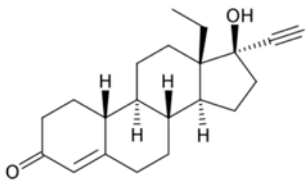
Keywords: liquid chromatography, surfactant, micellar mobile phase, short column, contraceptive drugs

1. INTRODUCTION

Oral contraceptives are medications that prevent pregnancy. They are a very effective method for birth control. Oral contraceptives are pharmaceutical formulations containing steroid hormones in a relatively small amount. Female sex hormones consist of estrogen and progestin. Therefore, oral contraceptives are hormonal preparations that may contain combinations of the hormones estrogen and progestin or progestin alone [1, 2].

More stable estrogens, such as ethinyl estradiol (EE) and progestogens (or progestins), such as levonorgestrel (LNG), gestodene (GES) and cyproterone acetate (CPA) are used more frequently for medical purposes. The structures of EE, CPA, GES and LNG are shown in Table 1. The prolonged use of the hormones cause long-term risks that are related to doses and to individual susceptibility [3]. Therefore, the effective and sensitive method of quantitative determination in pharmaceutical preparations is required.

Table 1. Structures and molecular weight (MW) of contraceptive drugs.

Contraceptive drugs	Structure	MW (g mol^{-1})
Ethinyl estradiol (EE)		296.403
Cyproterone acetate (CPA)		416.940
Gestodene (GES)		310.430
Levonorgestrel (LNG)		312.446

Various analytical methods have been developed for determination of contraceptive drugs in different matrices (including pharmaceutical preparations [1, 4-8], environmental samples [9, 10] and biological sample [11-16]) such as high performance liquid chromatography [1-5, 7, 9, 15-20], gas chromatography [12, 21], micellar electrokinetic capillary chromatography [22], capillary liquid chromatography and capillary electrochromatography [23]. HPLC techniques are commonly used for contraceptive drugs analysis [10].

Most HPLC systems for the determination of contraceptive drugs consist of an organic mobile phase (methanol and/or acetonitrile), C18 analytical column (100-250 mm length) and UV detection. The conventional analytical columns in HPLC are expensive and long, thus the analysis time is quite long. Moreover, the uses of organic mobile phases such as methanol and/or acetonitrile, etc., are not safe. For this reason, the traditional LC techniques used for the analysis of pharmaceutical consume tremendous amounts of toxic organic solvents and consequently generate large quantities of waste. Most of them are harmful to the environment and operators. Therefore, the green analytical method has gained increasing interest in pharmaceutical analysis. There are several approaches to achieve this aim such as using eco-friendly solvents, minimizing organic solvent consumption, reducing waste generation, reducing the chromatographic separation time, etc [24-27]. This proposed method implemented by using eco-friendly mobile phase and shortening the analytical column length to minimize hazardous solvent consumption and waste production.

In this work, SDS has been used as a micellar mobile phase with short column (C18, 12.5 mm length) instead of the expensive and conventional analytical column. The optimized method has been applied to determine EE, CPA, GES and LNG in oral contraceptive pills.

2. MATERIALS AND METHODS

2.1 Reagents and Chemicals

All chemicals and reagents used in this work were of analytical reagent (AR) grade. Ethinyl estradiol (EE) and gestodene (GES) were purchased from Sigma (China). Cyproterone acetate (CPA) was purchased from Sigma (Canada). Levonorgestrel (LNG), USP reference standard, was purchased from USP (Germany). Sodium dodecyl sulfate (SDS) was purchased from Carlo Erba (Italy). HPLC-grade methanol and 2-Propanol (Isopropanol, IPA) were purchased from Merck (Germany). Water was deionized and purified on an ultrapure water purification system (Siemens Water Technologies, USA) and used to prepare all solutions.

The oral contraceptive samples consisted of 13 commercial samples (different brands, Sample No. 1-13) with three formula of the binary compositions such as EE+CPA, EE+GES and EE+LNG. They were purchased from drugstores in Pathum Thani province, Thailand.

2.2 Instrumentation and Chromatographic Conditions

HPLC analyses were carried out making use of LC-30A Nexera liquid chromatograph (Shimadzu, Japan) consisting of a DGU-20A5 on-line degasser, a LC-30AD pump, a CTO-20AC column oven, a SIL-30AC autosampler, a SPD-M20A diode array detector (DAD) and a CBM-20A system controller. Data acquisition and processing were controlled by the LabSolutions software (Shimadzu, Japan). The short column Zorbax SB-C18 (12.5 × 4.6 mm i.d., 5 μm), Agilent Technologies (USA), was used as an analytical

column. The chromatographic conditions were 0.05 mol L^{-1} SDS and isopropanol (95:5, v/v) as micellar mobile phase with a flow rate of 1.0 mL min^{-1} and an injection volume of 20 µL. The detection wavelengths were 281, 281, 240 and 240 nm for EE, CPA, GES and LNG, respectively.

2.3 Standard Solution Preparation

The stock standard solutions of EE, CPA, GES and LNG were prepared at a concentration of 500, 4000, 500 and 500 µg mL^{-1} by accurately weighing 0.0255, 0.2041, 0.0255 and 0.0250 g, respectively and dissolving each of them in 10.0 mL methanol followed by dilution to 50.0 mL with deionized water. The stock solutions were stored in a refrigerator at 4 °C. The working standard solutions were prepared daily by further dilution of the stock solutions with a mobile phase and filtered through a 0.20 µm nylon syringe filter (Chromex Scientific, UK) before injecting into the HPLC system.

The stock SDS solution at a concentration of 0.5 mol L^{-1} was prepared by weighing 72.1225 g and then adding 400 mL deionized water. The solution was stirring using a magnetic stirrer (Nickel-Electro Ltd., UK) for 15 min until it was dissolved. The solution was further diluted to 500.0 mL with deionized water. The mobile phases were prepared at the desired concentration by diluting the proper amount of this stock solution. Finally, the solutions were filtered through a 0.20 µm nylon membrane filter (Chromex Scientific, UK).

2.4 Sample Preparation

To evaluate the performance of the proposed method, the commercial oral contraceptive tablets were analyzed. Twenty-one tablets were weighed and ground in a porcelain mortar to let it fine and homogeneous powder. An appropriate portion of powder equivalent to the mass of two tablets was accurately weighed and dissolved in 2.5 mL of methanol into 25.0 mL volumetric flask. This mixture was sonicated (CT Brand, China) for 15 min and then adjusted to the volume with the mobile phase. The content of the flask was sonicated again for 5 min to complete dissolution of the drug and then centrifuged (Gemmy Industrial, Taiwan) at 4000 rpm for 15 min. The clear supernatant was finally filtered through a 0.20 µm nylon syringe filter and 20 µL aliquot was injected into the HPLC system.

3. RESULTS AND DISCUSSION

3.1 Preliminary Study

For the preliminary study, the effect of organic modifier addition on the mobile phase was investigated. Organic modifier used in this method was isopropanol. The mobile phase containing 0 and 10 %v/v of isopropanol in 0.05 mol L^{-1} SDS was studied with Zorbax SB-C18 (12.5 × 4.6 mm i.d., 5 µm) as an analytical column by injecting each standard solution of EE, CPA, GES and LNG at concentration of 20 µg mL^{-1} at a flow rate of 1.0 mL min^{-1} and an injection volume of 20 µL.

The results showed that the addition of isopropanol into mobile phase affects retention time and peak shape of the analytes. The chromatograms are shown in Figure 1. The results demonstrate that the mobile phase containing isopropanol not only decrease retention time but also improve the peak symmetry. Therefore, isopropanol was selected for the chromatographic condition.

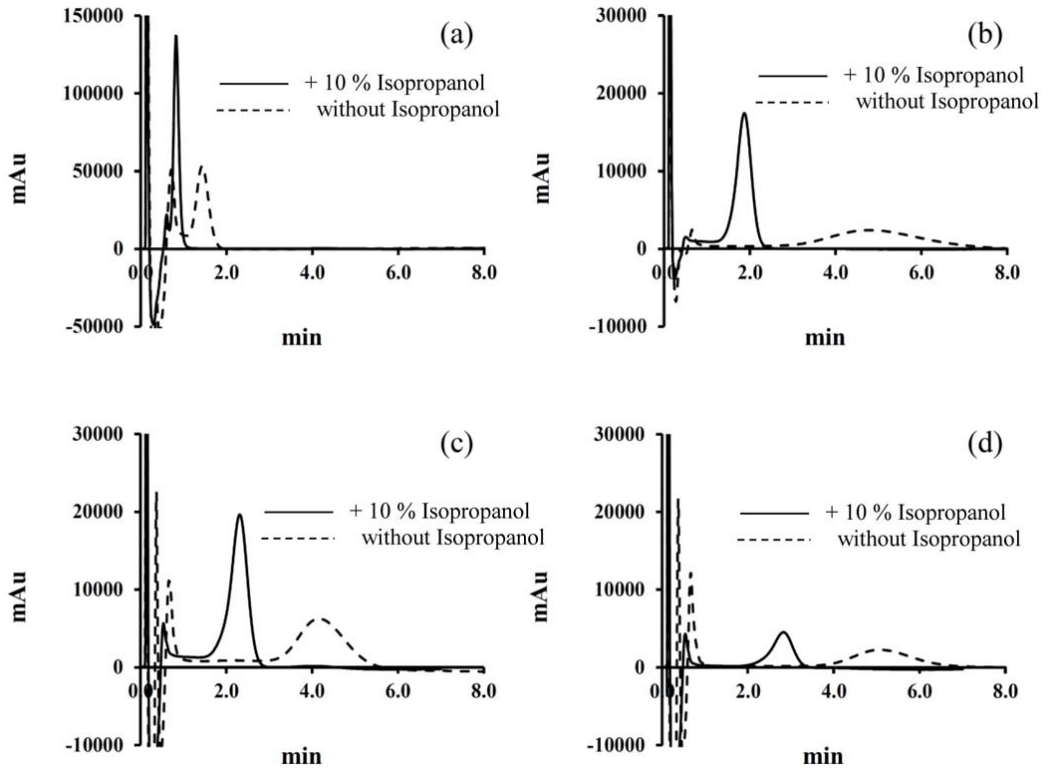


Figure 1. Chromatograms of (a) EE, (b) CPA, (c) GES and (d) LNG (each concentration 20 µg mL^{-1}) using mobile phase containing 0.05 mol L^{-1} SDS (---) with and (—) without isopropanol (10, v/v). Chromatographic condition; column: Zorbax SB-C18 (12.5 × 4.6 mm i.d., 5 µm), flow rate: 1.0 mL min^{-1}, injection volume: 20 µL.

3.2 Chromatographic Behaviors

Since a short analytical column has been used, the chromatographic behavior needs to confirm the performance of the system.

3.2.1 Chromatographic behavior of solutes in a micelle system

The chromatographic behavior of solutes in a micelle system with conventional analytical column C8 and C18 was reviewed [28], according to the equation 1.

$$1/k = C_0 + C_1 \cdot [M] \quad (1)$$

Where k is capacity factor ($k = (t_R - t_0)/t_0$), C_0 and C_1 are formal constants. [M] is the concentration of micelle in the mobile phase and [M] is equal to the difference between total concentration of surfactant, [S], and its critical micelle concentration, CMC ([M] = [S] - CMC).

For the study of the chromatographic behavior, concentrations of surfactant (SDS) at higher the critical micellar concentration (CMC of SDS is 8.27 mmol L^{-1}) were studied at 0.025, 0.05, 0.075, 0.10, 0.125 and 0.15 mol L^{-1}. The flow rate of mobile phase was 1.0 mL min^{-1} and the injection volume was 20 µL. EE, CPA and GES at a concentration of 20 µg mL^{-1} and LNG at a concentration of 5 µg mL^{-1} were injected into the system. The capacity factor of each contraceptive drug and the chromatographic behavior of solutes in a micelle system

were then calculated.

It was found that the reciprocal value of capacity factor (k) of EE, CPA, GES and LNG versus the concentration of micelle in the mobile phase [M] were linear, as shown in equation 2, 3, 4 and 5, respectively, and $R^2 > 0.99$ (Figure 2 (a)), corresponding to chromatographic behavior of solutes in a micelle system.

$$1/k_{EE} = 2.4943[M] + 0.0507 \quad (R^2 = 0.9911) \tag{2}$$

$$1/k_{CPA} = 1.0555[M] + 0.0168 \quad (R^2 = 0.9938) \tag{3}$$

$$1/k_{GES} = 1.0505[M] + 0.0062 \quad (R^2 = 0.9931) \tag{4}$$

$$1/k_{LNG} = 0.8935[M] + 0.0055 \quad (R^2 = 0.9940) \tag{5}$$

3.2.2 Chromatographic behavior of solutes in RP-HPLC system

The chromatographic behavior of solutes in RP-HPLC system with conventional analytical column C8 and C18 was over reviewed [29], according to the equation 6.

$$\log k = a_m - m_{hvb}\varphi \tag{6}$$

Where k is capacity factor ($k = (t_R - t_0)/t_0$), a_m and m_{hvb} are formal constants. φ is the volume fraction of organic modifier in the mobile phase.

For the study of the chromatographic behavior of solutes in RP-HPLC system, isopropanol was mixed with SDS and varied ratios at 0, 2.5, 5.0, 7.5, 10.0 and 12.5 %v/v. The chromatographic condition is described in Section 3.2.1. The capacity factor of each contraceptive drug and the chromatographic behavior of the solutes in RP-HPLC system were then computed.

The results showed that the log k of EE, CPA, GES and LNG were in a linear in relation to volume fraction (φ), as shown in equation 7, 8, 9 and 10, respectively, and $R^2 > 0.99$ (Figure 2 (b)), corresponding to chromatographic behavior of solutes in RP-HPLC system.

$$\log k_{EE} = -3.0253\varphi + 0.9714 \quad (R^2 = 0.9899) \tag{7}$$

$$\log k_{CPA} = -3.4899\varphi + 1.4316 \quad (R^2 = 0.9555) \tag{8}$$

$$\log k_{GES} = -2.1722\varphi + 1.4310 \quad (R^2 = 0.9695) \tag{9}$$

$$\log k_{LNG} = -2.0805\varphi + 1.4955 \quad (R^2 = 0.9441) \tag{10}$$

As has been described in Section 3.2.1 and 3.2.2, the results demonstrate that it is possible to use the short column, Zorbax SB-C18 (12.5 × 4.6 mm i.d., 5 µm), as an analytical column for the separation of contraceptive drugs.

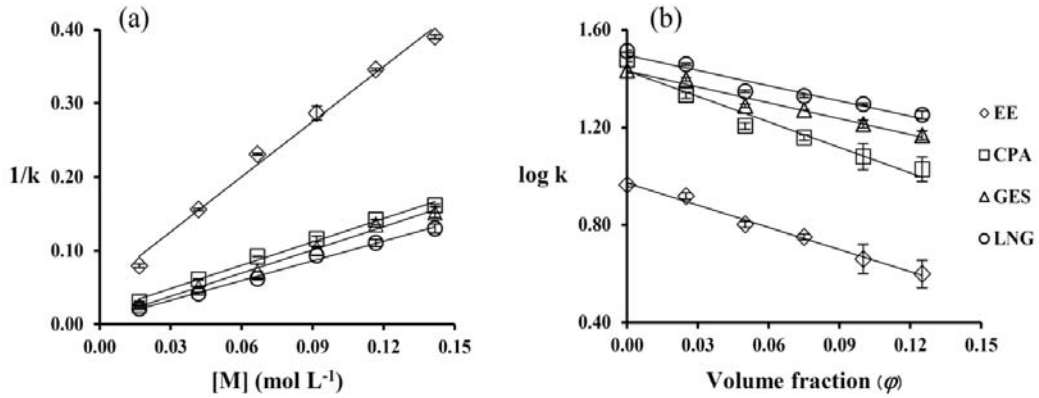


Figure 2. The relationship between (a) the reciprocal value of capacity factors of each analyte (1/k) and the concentration of surfactant as micelle foam in mobile phase ([M]) and (b) the log k and the volume fraction (φ) of organic modifier in the mobile phase.

3.3 Optimization of the Chromatographic Condition

The chromatographic conditions such as compositions of mobile phase (organic modifier and concentration of micellar mobile phase) and flow rate were optimized making use of univariate optimization. Initially, Zorbax SB-C18 (12.5 × 4.6 mm i.d., 5 mm) has been used as an analytical column at a flow rate of 1.0 mL min^{-1} and the injection volume was 20 µL.

3.3.1 Compositions of mobile phase

3.3.1.1 Effect of organic modifier

As described in Section 3.1, the use of increased concentrations of isopropanol with reverse phase column leads to significant decrease in retention time and also improve the symmetric of peaks. Therefore, isopropanol content in mobile phase was optimized. The amount of the modifier was varied in the range of 0-12.5 %v/v. The results showed that the retention time of EE, CPA, GES and LNG peaks decreased when organic modifier increased, as shown in Figure 3 (a). The addition of higher amount of isopropanol led to shorter retention times and worsened the separation. Therefore, the mobile phase containing 5 %v/v of isopropanol was selected as an optimum for the proposed method.

3.3.1.2 Effect of concentration of micellar mobile phase

The concentration of surfactant (SDS) in mobile phase has a key effect on chromatographic separation. In this method, the surfactant (SDS) amounts exceeding the critical micellar concentrations were varied in the range of 0.025-0.15 mol L^{-1}. It was found that the retention time of EE, CPA, GES and LNG decreased when the concentration of SDS increased, as clear in Figure 3 (b). Thus, SDS at a concentration of 0.05 mol L^{-1} was chosen for the developed chromatographic method with satisfied separation efficiency and analytical time.

3.3.2 Flow rate

The flow rate in HPLC systems affects the migration time of the analytes. Increasing flow rate usually cause faster elution. In this work, the effect of diffident flow rate at 0.6, 0.8, 1.0 and 1.2 mL min^{-1} (Figure 3 (c)) was examined. The results showed that flow rate at 1.0 mL min^{-1} was selected for the proposed method. These conditions were found to achieve the complete separation within 5 min.

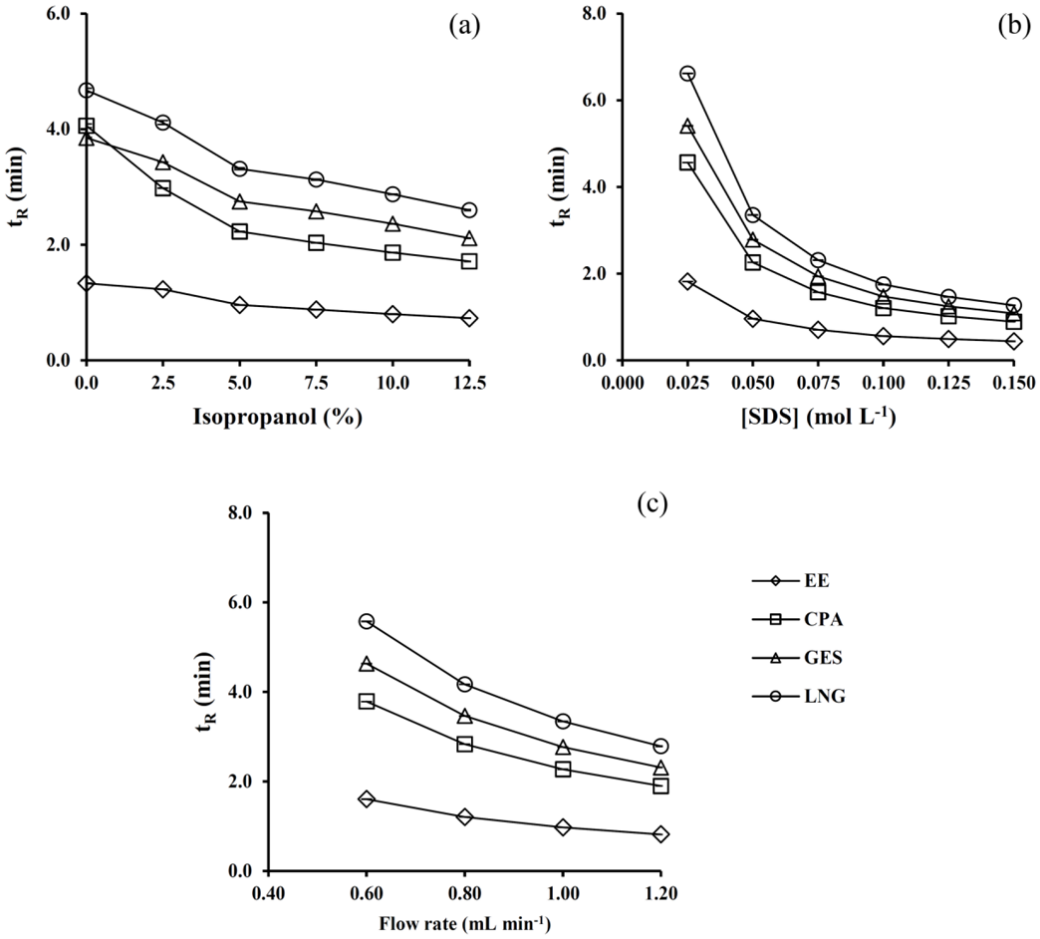


Figure 3. The optimization of the chromatographic condition (a) the effect of organic modifier, (b) the effect of concentration of micellar mobile phase and (c) the effect of flow rate. Chromatographic condition; column: Zorbax SB-C18 (12.5 × 4.6 mm i.d., 5 µm), injection volume: 20 µL.

3.4 Study of Some Analytical Features

The linearity, limit of detection (LOD), limit of quantification (LOQ), repeatability, reproducibility and recovery were examined for validation of the method.

3.4.1 Linearity

The working range of the method was studied by performing three replicate injections at eight standard concentrations of EE, CPA, GES and LNG. The calibration curves were constructed from peak area versus concentration of each analyte. The results showed that the working range of the method of EE, CPA, GES and LNG were 0.5-60, 1-1200, 1-60 and 1-60 µg mL^{-1}, respectively and R^2 of each analyte were higher than 0.9990 (Table 2).

3.4.2 Limit of detection (LOD) and quantification (LOQ)

The detection and quantification limits were studied (3S/N and 10S/N, respectively). The limits of detection were 0.003, 0.06, 0.025 and 0.05 µg mL^{-1} for EE, CPA, GES and LNG, respectively. The limits of quantification were 0.01, 0.20, 0.08 and 0.16 µg mL^{-1} for EE, CPA, GES and LNG, respectively.

The results are illustrated in Table 2.

3.4.3 Repeatability and reproducibility

To determine method precision, standard solutions (30 µg mL^{-1} of EE, GES and LNG and 600 µg mL^{-1} of CPA) were injected into the system ten times using the same standard solution. The results are shown in Table 2. In all instances, the RSD values of retention time and peak area response were less than 0.3%, indicating excellent repeatability of the assay.

Reproducibility of analysis was studied by selecting 3 different formulation samples (Sample No. 1, Sample No. 9 and Sample No. 11). The sample solutions were extracted with mobile phase and then analyzed, as described in Section 2.4. In all instances, the RSD values of the amount of contraceptive drugs in sample solutions were less than 2.0% (the results were summarized in Table 2). The values of RSD obtained for the amount of contraceptive drugs proves the good reproducibility of the method.

3.4.4 Recovery study

Recovery study was carried out by spiking three samples (Sample No. 1, 9 and 11) with three known amounts at 50, 100 and 150% of labeled amount of each analyte. The results showed that the recoveries of Sample No. 1, 9 and 11 were in the range of 98-102% (Table 2). Therefore, the recovery percentage proved that the method was sufficiently accurate within the desired range.

Table 2. Some analytical features of the proposed method.

Analytical features	EE	CPA	GES	LNG
Linearity				
- Range (µg mL^{-1})	0.5-60	1-1200	1-60	1-60
- Slope	56992	25851	33314	29359
- Intercept	-7269	-233986	-26888	-12786
- R^2	0.9998	0.9995	0.9993	0.9995
LOD (3S/N) (µg mL^{-1})	0.003	0.06	0.025	0.05
LOQ (10S/N) (µg mL^{-1})	0.01	0.20	0.08	0.16
Repeatability (%RSD, n=10)				
- Retention time	0.26	0.12	0.12	0.15
- Peak area	0.04	0.05	0.17	0.14
Reproducibility (%RSD, n=3)				
- Sample No. 1	0.45	2.0	-	-
- Sample No. 9	1.25	-	0.24	-
- Sample No. 11	0.94	-	-	0.35
Recovery* (%)				
- Sample No. 1	98.79-99.36	98.17-99.52	-	-
- Sample No. 9	99.76-100.49	-	98.02-100.85	-
- Sample No. 11	99.49-101.27	-	-	99.25-101.30

* spiked each of analyte with three concentration levels into three sample solutions.

3.5 Analysis of Samples

To demonstrate the applicability of the proposed method, it has been used for the analysis of the commercial oral contraceptives. The sample solutions were prepared and then analyzed, as described in Section 2.4. Each sample was determined in triplicate. The chromatograms of the standard at optimal condition are shown in Figure 4. The amounts of each analyte found in the samples and %label amount are summarized in Table 3. The results indicated that all samples were within the USP range of 90.0-110.0% of the labeled amount [30].

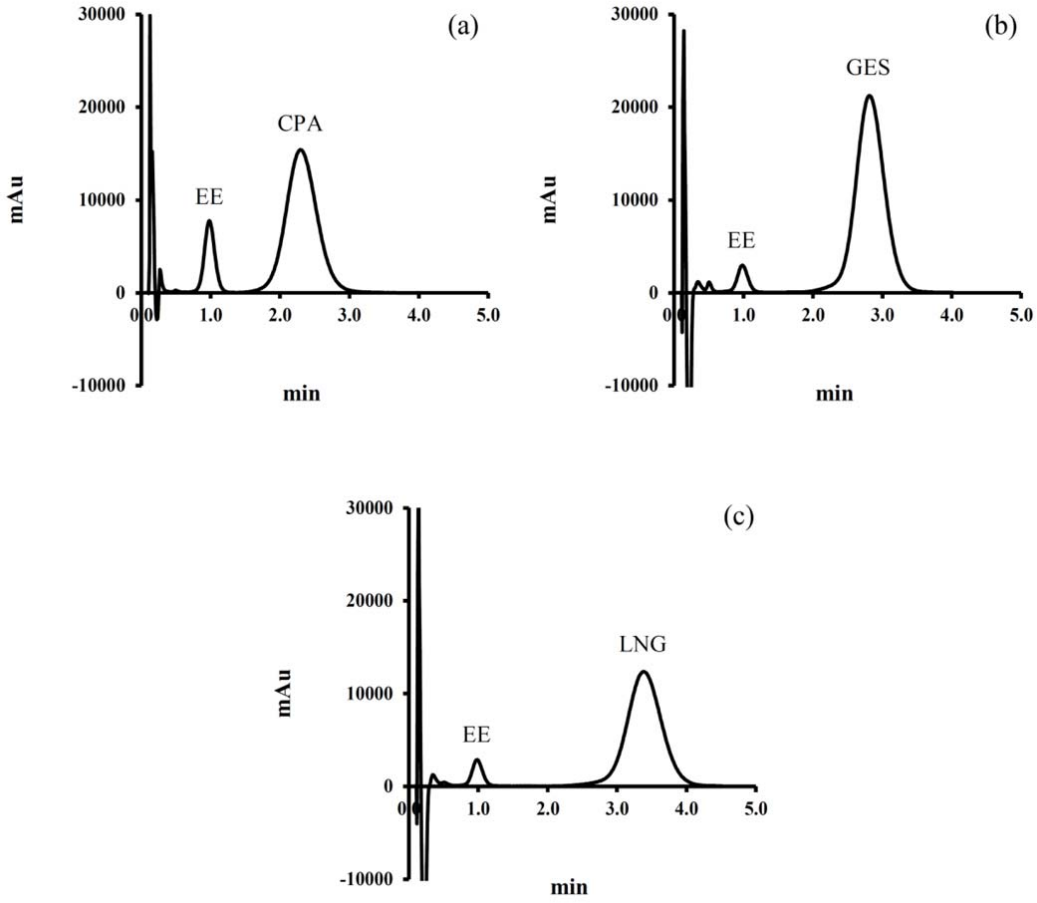


Figure 4. Chromatograms of (a) EE and CPA, (b) EE and GES, and (d) EE and LNG. Chromatographic condition; column: Zorbax SB-C18 (12.5 × 4.6 mm i.d., 5 µm), mobile phase: 0.05 mol L^{-1} SDS and isopropanol (95:5, v/v), flow rate: 1.0 mL min^{-1}, injection volume: 20 µL, detection wavelengths: 281, 281, 240 and 240 nm for EE, CPA, GES and LNG.

Table 3. Analysis of EE, CPA, GES and LNG quantity in oral contraceptive pills.

Sample No.	Analytes	Labeled (µg/tb)	Average found±SD* (µg/tb)	Average % label amount±SD*
1	EE	35	35.39±0.16	101.1±0.5
	CPA	2000	1961±40	98.0±2.0
2	EE	35	34.43±0.42	98.4±1.2
	CPA	2000	1957±1	97.9±0.1
3	EE	35	35.09±0.25	100.3±0.7
	CPA	2000	2034±13	101.8±0.7
4	EE	35	35.06±0.33	100.2±0.9
	CPA	2000	2022±29	101.1±1.5
5	EE	35	34.85±0.08	99.6±0.2
	CPA	2000	1974±29	98.7±1.5
6	EE	35	34.68±0.26	99.1±0.7
	CPA	2000	1974±34	98.7±1.7
7	EE	15	15.20±0.27	101.3±1.8
	GES	60	61.18±0.68	102.0±1.1
8	EE	15	15.27±0.04	101.8±0.3
	GES	60	60.48±0.32	100.8±0.5
9	EE	20	19.66±0.25	98.3±1.2
	GES	75	75.10±0.18	100.1±0.2
10	EE	20	19.78±0.37	98.9±1.9
	GES	75	74.78±0.20	99.7±0.3
11	EE	30	29.99±0.28	100.0±0.9
	LNG	150	150.4±0.5	100.3±0.4
12	EE	30	30.16±0.42	100.5±1.4
	LNG	150	148.8±1.1	99.2±0.7
13	EE	30	30.16±0.31	100.5±1.0
	LNG	150	152.6±1.0	101.8±0.7

* SD = standard deviation (n=3).

This proposed method with a short column (12.5 mm length) and an environmental friendly mobile phase was compared to the published HPLC methods for the determination of some contraceptive drugs in oral contraceptives [2, 4, 7, 19, 31-32]. The parameters that were compared include chromatographic conditions, some analytical features and solvent consumption as shown in Table 4. The advantage of this proposed method over other HPLC methods is the reduction of waste generation (more than 10 times) with short analysis time. Moreover, no organic solvent was used in the sample preparation process and the cost of the short column is cheaper than the conventional column (125-250 mm length).

Table 4. Comparative review of some contraceptive drugs analysis in oral contraceptives by LC techniques.

Year	Column	Mobile phase	Flow rate (mL min^{-1})	Detection[a]	Analysis time (min)	Solvent consumption[b] (mL)	Analyte	Linearity (µg mL^{-1})	LOD (µg mL^{-1})	Ref.
2004	C18 (150×4.6 mm, 5 µm)	methanol:water (80:20)	1.0	UV	6	48	Ethinyl estradiol Gestodene	5-80 10-200	0.8[c] 2.3[c]	[7]
2008	C18 (150×4.6 mm, 4 µm)	acetonitrile: methanol:water (30:20:50)	1.0	UV	20	30	Ethinyl estradiol Levonorgestrel Gestodene	2.4-60 12-300 9-160	- - -	[2]
2013	C18 (250×4.6 mm, 5 µm)	acetonitrile: water (80:20)	1.0	UV	7	48	Ethinyl estradiol Levonorgestrel	4-25 20-125	0.032[c] 0.066[c]	[4]
2013	C18 (125×4.0 mm, 5 µm)	acetonitrile: water (50:50)	1.0	Fluorescence UV	7	30	Ethinyl estradiol Drospirenone	0.1-10 15-1500	0.02[c] 4.88[c]	[19]
2016	C18 (150×4.6 mm, 5 µm)	acetonitrile: water (50:50)	1.0	UV	8	30	Ethinyl estradiol Drospirenone Gestodene Levonorgestrel	0.622-1.866 60.0-180.0 1.512-4.536 3.0-9.0	0.028[c] 9.500[c] 0.132[c] 0.763[c]	[31]
2016	C18 (150×4.6 mm, 5 µm)	acetonitrile: methanol:water (60:15:25)	1.0	UV	4	45	Ethinyl estradiol Levonorgestrel	2-14 1-70	0.03[d] 0.84[d]	[32]
The purposed method	C18 (12.5×4.6 mm, 5 µm)	0.05 mol L^{-1} SDS: isopropanol (95:5)	1.0	UV	5	3	Ethinyl estradiol Cyproterone Gestodene Levonorgestrel	0.5-60 1-1200 1-60 1-60	0.003[e] 0.06[e] 0.025[e] 0.05[e]	-

[a] UV: Ultraviolet detection, MS: Mass spectrometry

[b] Organic solvent in mobile phase per hour

[c] The limit of detection (LOD) was an estimation of 3.3(s/S); where s is the standard deviation of y-intercept and S is the slope of the calibration curve.

[d] The limit of detection (LOD) was an estimation of 3(s/S); where s is the standard deviation of y-intercept and S is the slope of the calibration curve.

[e] The limit of detection (LOD) was an estimation of 3S/N.

4. CONCLUSIONS

This work proposed the use of a short column (Zorbax SB-C18) as an analytical column with an environmental friendly mobile phase for the determination of binary mixer of contraceptive drugs such as EE, CPA, GES and LNG. Under the optimum condition, the proposed method was successfully applied for the determination of contraceptive drugs in commercial oral contraceptive pills. The results indicated that all samples were within the requirement of USP. The proposed method present a green analytical liquid chromatography has the potential of cost effective analysis, reduced chemical consumption and minimum waste generation.

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