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Research paper

Quantification of selected corticosteroids in herbal products

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ABSTRACT

Context: Corticosteroids are a group of synthetic drugs that are very close in structure to cortisol. ***Objective:*** In this study, our focus is to optimize the HPLC method for several corticosteroids that might be added up in consumers' products, including tablets and capsules illegally. ***Methods:*** The corticosteroids were well separated by using the HPLC method of the gradient solvent system, in which the mobile phase consisted of acetonitrile, water, and methanol at a 1 mL/min flow rate and a column temperature of 40°C. The optimized HPLC method was validated based on the LOD, LOQ, linearity, recovery, repeatability, and precision in both product matrices. The parameter of extraction efficiency and matrix effect was also investigated. ***Results****:* The matrix effect investigation on different products of tablets and capsules showed that the matrix did not affect the analysis. All studied corticosteroids showed linearity in the range of 2-24 mg/mL with the R^2 value between 0.97 and 0.99. The recovery was between 88.20 and 119.50%, with LOD 0.37-0.73 mg/mL and LOQ 0.89-1.74 mg/mL. The intermediate precision for both product matrices was satisfied. The second extraction in the extraction efficiency investigation gave less than 10% from the first extraction. *Conclusion:* The developed HPLC test method could be used to analyze the presence and quantity of the studied corticosteroids. The sample preparation was optimized successfully, and it was indicated by the good recovery value.

Keywords: corticosteroids, HPLC, optimization, validation, herbal product

INTRODUCTION

Corticosteroids are hormone mediators produced by the cortex of adrenal glands that are further categorized into glucocorticoids, mineralocorticoids, and androgenic sex hormones (Yasir et al., 2023). Glucocorticoids, sometimes referred to as corticosteroids, and their synthetic analogues have strong immunosuppressive properties, thus making synthetic glucocorticoids one of the top 50 World Health Organization essential medicines (Cheng et al., 2017). Prednisone, dexamethasone, and triamcinolone are used to treat a wide range of (auto)inflammatory conditions as well as hematopoietic malignancies. Other synthetic glucocorticoids were betamethasone, prednisolone, cortisone, and

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hydrocortisone. Synthetic glucocorticoid, however, has many side effects that usually involve homeostasis and tissue maintenance. Thus, any drug or steroid should be consumed only under the psychiatrist's prescription. Concerning the safety of the traditional product's consumer, Malaysia, through their regulatory agency, the Malaysia National Pharmaceutical Regulatory Authority (NPRA), is emphasizing the prohibition of selected substances, such as corticosteroids, in the products.

The monitoring and detection method for corticosteroids should be using one of the separation techniques, such as HPLC, GCMS, and LCMS. In our study, we develop the HPLC testing method to screen the presence of selected corticosteroids, inclusive of dexamethasone, prednisone, prednisolone, cortisone, triamcinolone, and betamethasone (Figure 1), in herbal products of tablets and capsules. This method covers the HPLC parameters and sample preparation. The matrix background from several traditional products of the two matrices was investigated in the initial stage. The chemical profile of the products shows no significant peak in the range of analyzed corticosteroid retention time. It could thus be used as the matrix in this study specifically for method validation and for sample preparation. We demonstrate that this method is simple and fast to monitor, detect, and screen the presence of the selected corticosteroids in tablet and capsule dosage forms of herbal products.

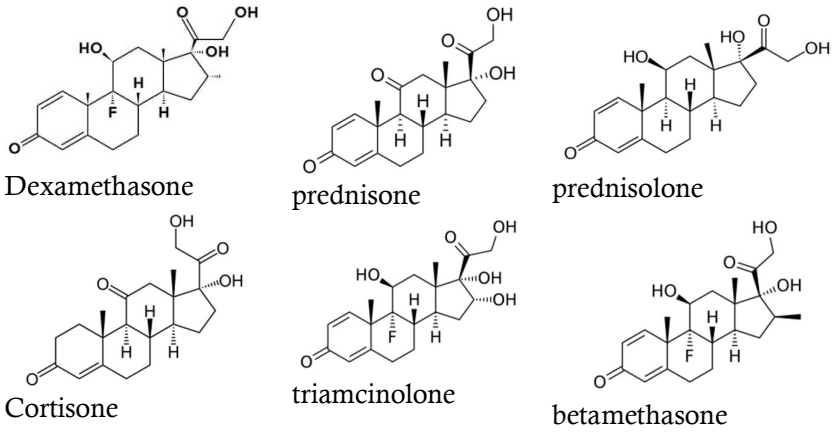

Figure 1: Chemical structure of selected corticosteroids.

METHODS

Standards and chemicals

The gradient grade of methanol and acetonitrile for sample preparation and HPLC analysis was purchased from Merck (Merck, Darmstadt, Germany). The chemical standards of dexamethasone, prednisone, prednisolone, cortisone, triamcinolone, and betamethasone were all purchased from Chromadex. The tablet and capsule for analysis were obtained from the commercial sample.

Preparation of standard solution

The stock of standard solution was prepared by firstly dissolving the reference standard with methanol and following with water in the ratio of 5:5 (v/v). The

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standards of corticosteroids were each prepared at 1000 mg/mL before being mixed together with the known final concentration for each standard in the solution. The working standard solutions were later prepared by diluting the stock solution into 5-25 mg/mL concentrations. The prepared working standard solutions were used to plot the standard calibration curve for quantification of the corticosteroids during method validation and sample extraction optimization.
The stock and working standard solution were kept at room temperature only. Data from the stability study shows very low degradation for each corticosteroid's standard within the 5-month observation.

HPLC method
The HPLC system used was Waters model E2605, comprising a quaternary pump system, a column heater SMH (H13 SMH 242 G), a PDA detector 2998, and a degasser. Development and optimization of the HPLC parameters were performed in the initial stage by using Sun Fire from Waters and the Luna column from Phenomenex. Both columns gave low resolution between peaks of the corticosteroids analyzed. The subsequent trial was using the hybrid column YMC-Triart, which gave good separation and resolution for all corticosteroids. All columns used in this study were 5 µm particle size, 4.6 mm diameter, and 250 mm length. Other parameters were also optimized, including temperature, mobile phase, and finally flow rate. The optimized HPLC method was composed of a temperature of 40°C, a mobile phase with a combination of water, acetonitrile, and methanol, and a flow rate of 1.0 mL/min.

HPLC method validation
To assess the analytical performance of the method, five parameters were analyzed for method validation, listed as linearity, recovery, limit of detection (LOD), limit of quantification (LOQ), repeatability, and precision.

Linearity
Linearity means a linear relationship between analyte signals and analyte concentrations in samples containing matrix components (ICH Guidelines, 2022). The sample of capsule or tablet with known concentration of corticosteroids (5, 10, 15, 20, 25 mg/mL) was analyzed by using the optimized HPLC method. The experimental value of the concentrations was later plotted to gain the linearity value of the method, R^2. At least three different individual replicates were analyzed for each concentration.

Recovery
Recovery calculation is used to determine losses that can occur during the sample preparation process. The recovery value could be calculated by using this formula:

$$\%R=[(CF-CU)/CA] \times 100$$

CF=Experimental analyte concentration (calculation)
CU=Analyte concentration in the blank sample (blank matrix)
CA=Known analyte concentration (theory)

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Limit of detection (LOD)

LOD (limit of detection), is the lowest quantity of a substance that can be distinguished from the absence of that substance. The limit of detection (LOD) was calculated from the slope of the calibration curve and standard deviation of the concentrations of triplicate injections, using the following formula (ICH Guidelines, 2022):

$$LOD = [3 \times SD] + mean$$

Limit of quantification (LOQ)

Limit of Quantification (LOQ) is the lowest analyte concentration that can be quantitatively detected. The limit of detection (LOD) was calculated from the slope of the calibration curve and standard deviation of the concentrations of triplicate injections, using the following formula:

$$LOD = [10 \times SD] + mean$$

Precision

The evaluation for precision was carried out with intra and inter day experiments. Statistical value was relative standard deviation (RSD, %) as indicator of the precision or dispersion of the readings relative to the mean. The medium concentration of 10 mg/mL was selected for sample preparation in this evaluation. The experiment was carried out in two different days.

Sample preparation optimization

Sample preparation was using ultra sonication method. Tablet or capsule sample were firstly spiked with standard solution before add with solvent followed by sonication. After being sonicated, the vials then were centrifuged at 10,000 rpm. The supernatant liquid was filtered with syringe filter 0.45µm. Several parameters were analyzed in the optimization process such as time (5,10,15 minutes) and solvent (water, methanol and water methanol 5:5, v/v).

Data analysis

The HPLC data from chromatogram were transferred, analyzed and plotted by using Microsoft excel.

RESULTS

Method development

Three different columns were analysed in the initial stage. The columns used were SunFire by Waters, Luna by Phenomenex, and finally TYMC-Triart by TYMC. The HPLC chromatograms for each column are shown in figures 2a, 2b, and 2c. The TYMC column showed high resolution compared to the other two columns. The flow rate was initially set at 1.2 mL/min; however, it was changed to 1.0 mL/min to get better resolution. The increment of column temperature from room temperature to 40°C was found to fully resolve all peaks in the chromatogram (figure 2d). The advantage of TYMC-Triart was its hybrid properties between the organic and inorganic silica that emphasize versatility. This property enables it to withstand high operating pressure, temperature, and resistance to 100% water as a mobile phase without affecting the column matrix. The UV spectra were recorded

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by the PDA detector from 190 to 400 nm. The 245 nm gave an optimized peak observation and thus was selected to analyse and quantify the corticosteroids. The UV spectrum for each corticosteroid was displayed in figure 3, showing a similar profile but with different lambda max values. The final mobile phase was a combination of water, methanol, and acetonitrile with a gradient system. The gradient system was as in table 1. The quantification was carried out by using the calibration curve for each corticosteroid and linear equation. The calibration curve was plotted with the concentration range of 5-25 mg/mL (figure 4). The R^2 for each standard was more than 0.995. In order to investigate the background of the traditional product in the capsule and tablet dosage form, 10 different commercial products were analyzed as in figure 5. The HPLC profile of the products did not show any significant peak in the range of 14 minutes and above, which is the range of corticosteroid peaks observed. It has thus allowed the sample preparation method optimization and the HPLC method validation.

Sample preparation

Parameters that affect the extraction of corticosteroids from tablets and capsules were investigated. Figure 6 and 7 shows the result of correlation between the concentration which represent by the peak area with the time for sonication and different water methanol combination, for every corticosteroid. The amount of sample, solvents, and temperature has been set at a constant value. The optimization result for capsules was as shown in figure 6. The sonication times of 5, 10, 15, 20, 25, and 30 minutes indicated not much difference in the concentration except for dexamethasone, which gave the highest concentration at 15 minutes. The solvent of 50% water: methanol gave the highest concentration yield for all corticosteroids from the capsules. The result for tablets preparation shown as in figure 7. The graph for all standards shows similarities in the correlation between the extracted corticosteroids with the two studied parameters. It is obviously the optimum time, and solvents were 20 minutes and 50% water: methanol, respectively.

HPLC method validation

In our investigation, two sets of data for each tablet and capsule were prepared. The correlation graphs between concentration in the range of 5-25 mg/mL and peak area were plotted. The correlation coefficient values, R^2, were listed as in Table 2. The values were all close to 1 and thus show the linearity within the studied range. The concentrations were calculated by using equations in table 2, where x is the standard concentration for each corticosteroid and y is the peak area in the HPLC chromatogram.

The limit of detection (LOD) and limit of quantification (LOQ) were calculated by using equations 2 and 3. In the determination of LOD and LOQ for each corticosteroid in tablet and capsule, the values were listed in table 3. It could be observed that the LOD values for all corticosteroids in both matrices were in the range of 0.37-0.73 mg/mL. The values for LOQ were in the range of 0.89-1.74 mg/mL. Since the value of the minimum concentration of the working range is 5 ppm, which is higher than the highest LOQ value, the minimum value in the working range is acceptable for analysis.

The linearity determination was carried out by using concentrations of 5, 10, 15, 20, and 25 mg/mL to determine the correlation coefficient as the linearity parameter (Table 3). If the correlation coefficient is close to 1, it indicates good

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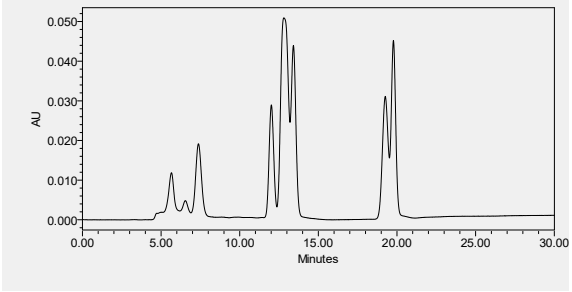
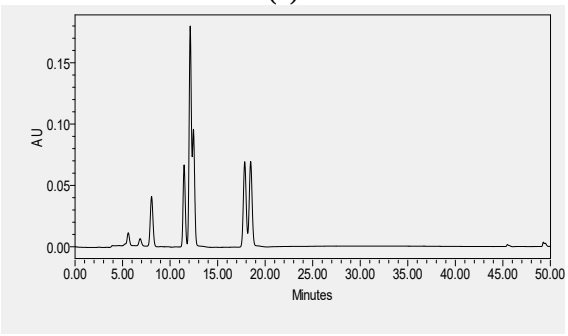
linearity between the study factors. The experiments were performed on at least six different



(a)



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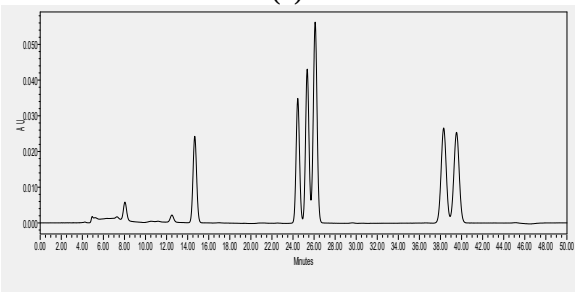

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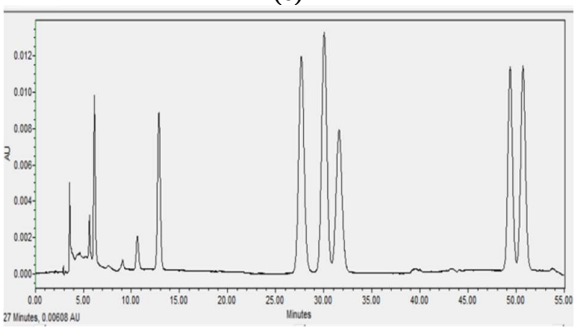

(d)

Figure 2: HPLC-PDA chromatogram of a standard mixture of corticosteroids using different columns

(a) SunFire by Waters (b) Luna by Phenomenex, (c) TYMC-Triart by TYMC, and (d) TYMC-Triart at 40°C and 1 m/min

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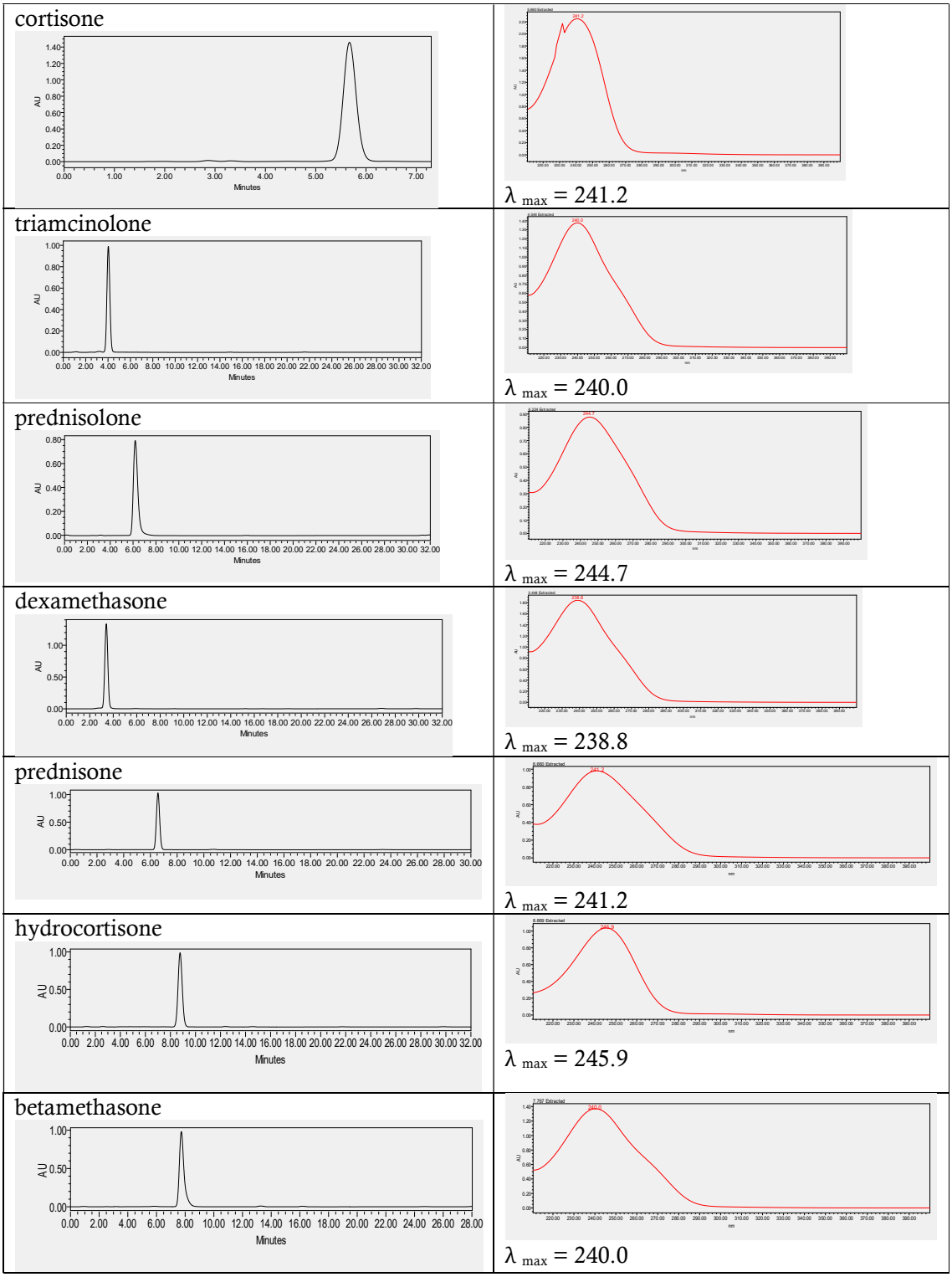

Figure 3: The UV spectrum of the corticosteroids with different lambda max values.

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Table 1: Gradient solvent system of HPLC method for standard mixture of studied corticosteroids

Time (min)	% acetonitrile	% water	% methanol
0	10	80	10.0
0.1	10	50	40.0
2.0	10	50	40.0
5.0	22	77	1.0
15.0	22	77	1.0
35.0	22	77	1.0
36.0	27	72	1.0
39.0	27	72	1.0
40.0	27	70	3.0

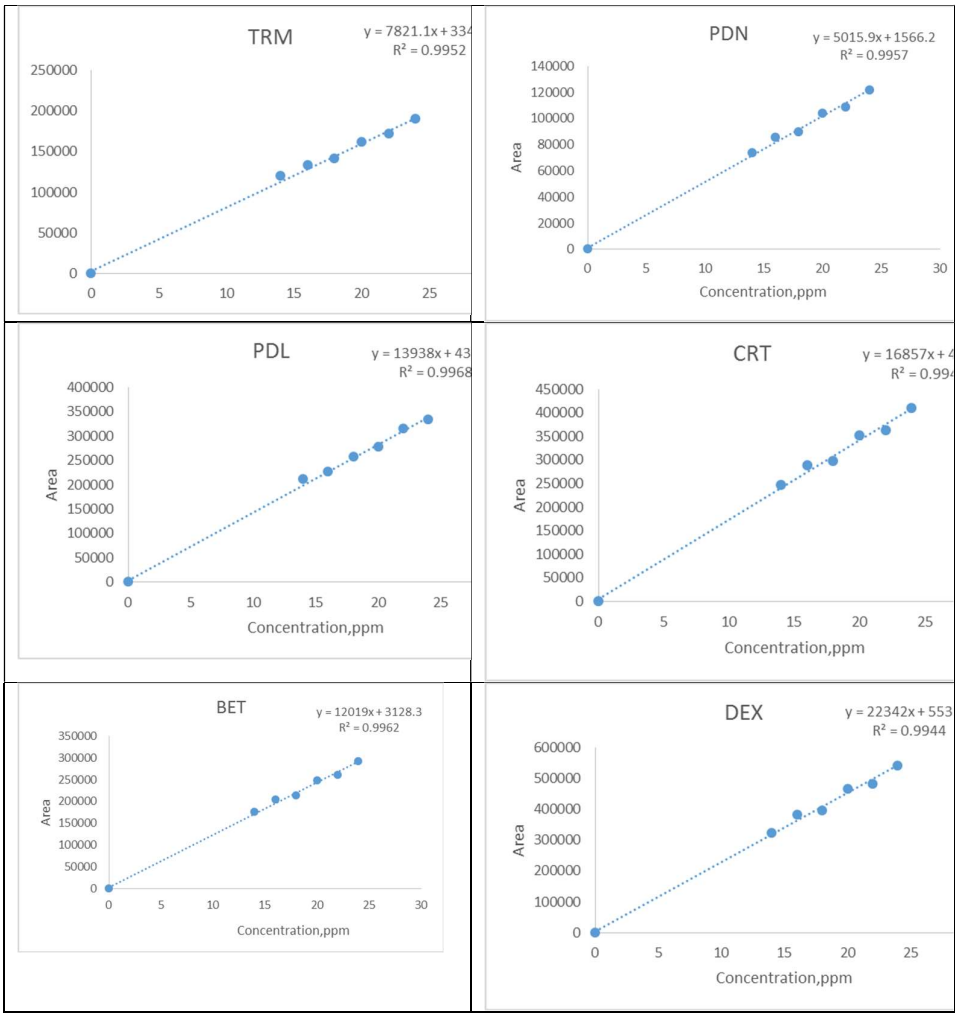

Figure 4: Calibration curve of standard corticosteroids.

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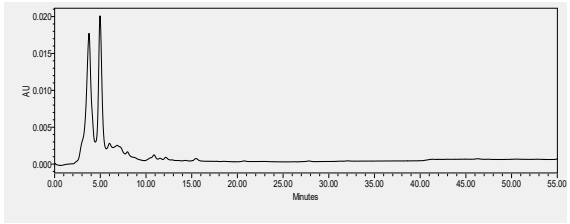
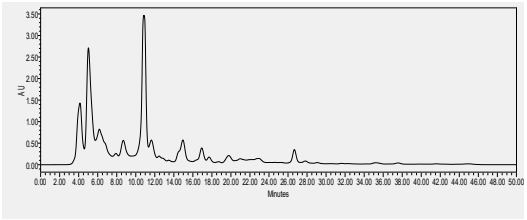
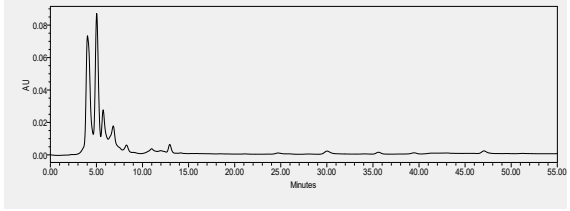


Tablet 1



Capsule 1



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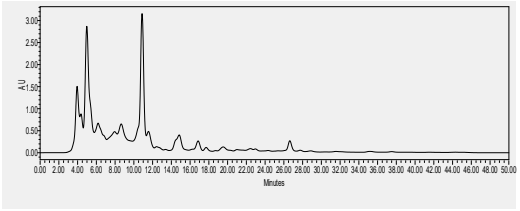
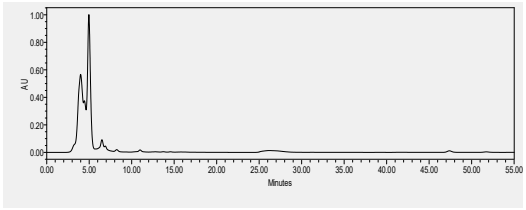
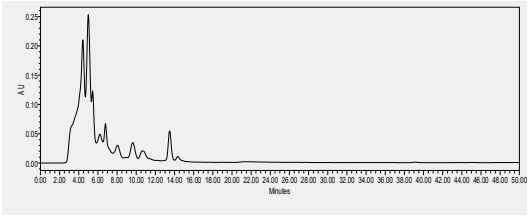


Capsule 2

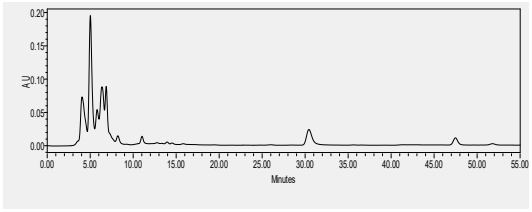


Tablet 3

J1



Capsule 3



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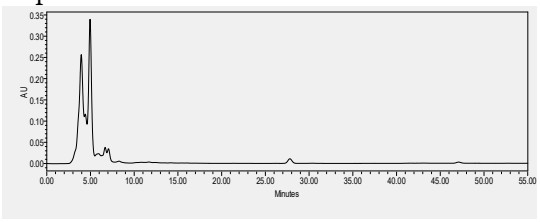

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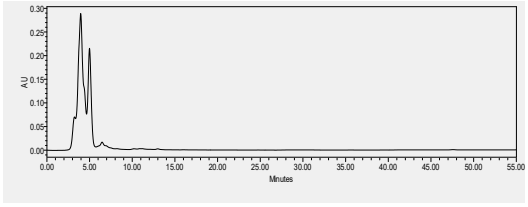
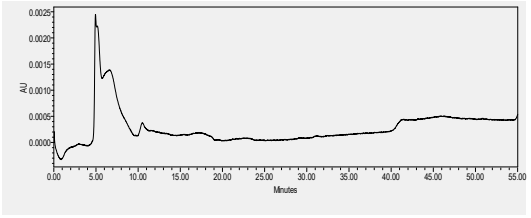


Tablet 5



Capsule 5

Figure 5: The chromatogram of commercialized tablet and capsule.

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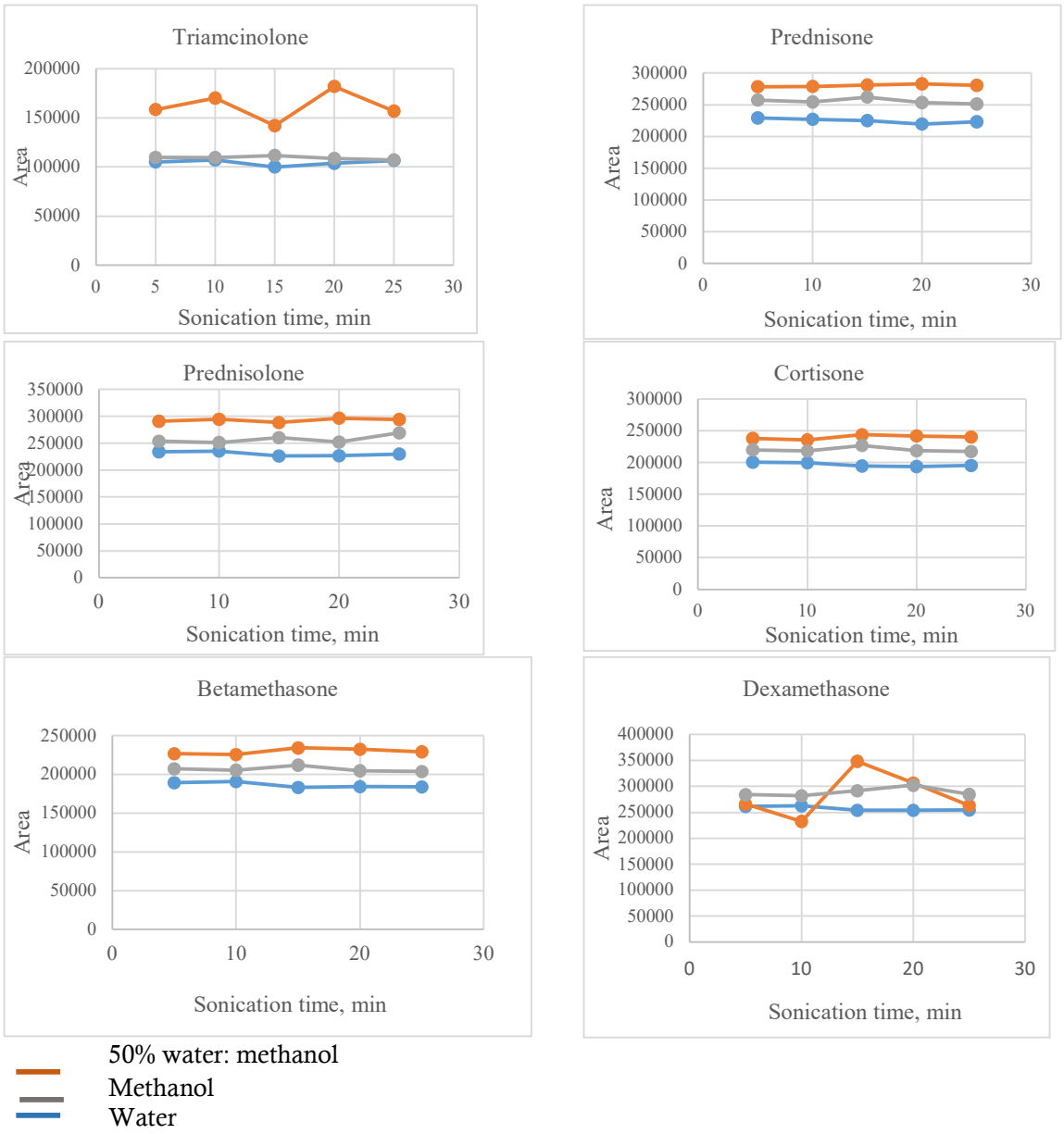

Figure 6: Result of correlation between the concentration which represent by the peak area with the time for sonication and different water methanol combination in capsule.

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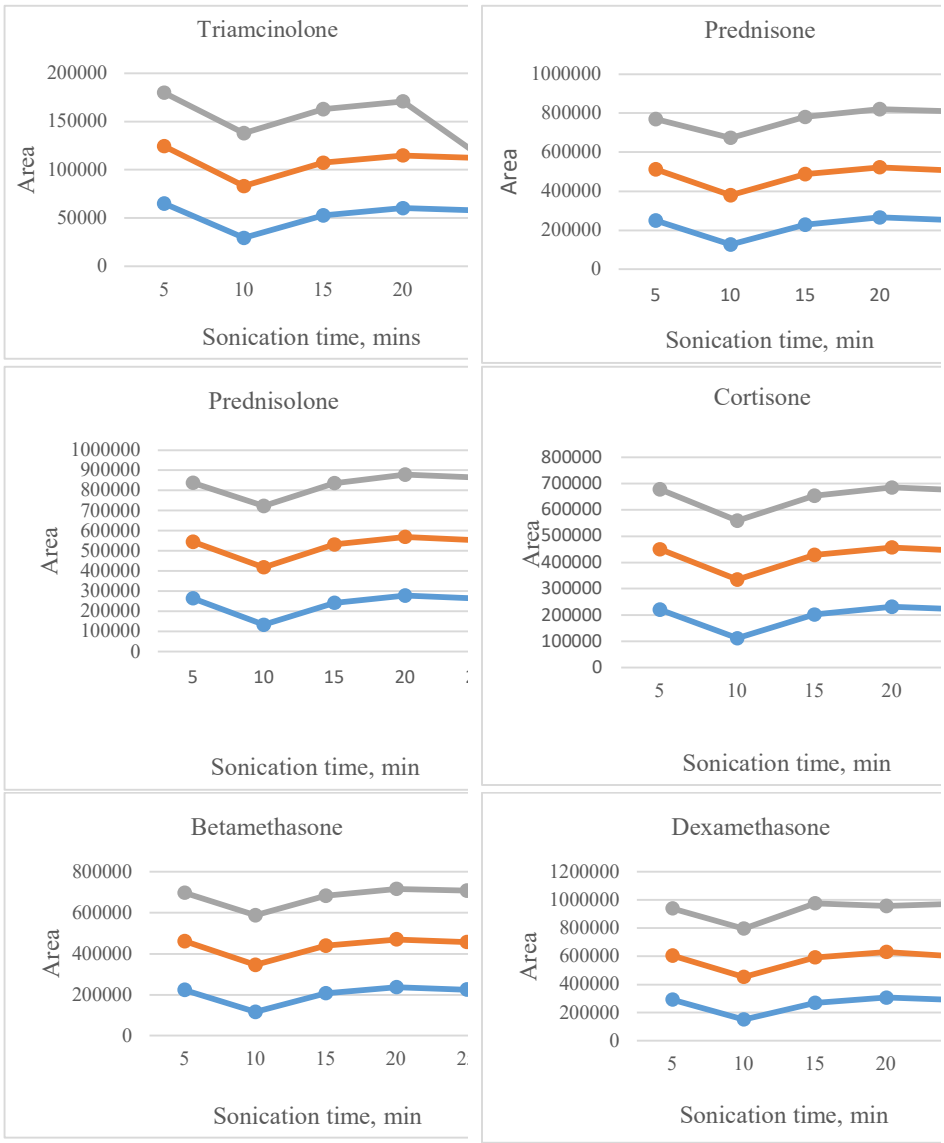

Figure 7: Result of correlation between the concentration, which is represented by the peak area, with the time for sonication and different water-methanol combinations in the tablet.

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Table 2: The linear equation for standards of corticosteroids and its correlation coefficient

Corticosteroids	Linear equation	Correlation coefficient, R^2
Triamcinolone	y = 11742x + 12019	0.9930
Prednisone	y = 18780x + 13822	0.9993
Prednisolone	y = 19591x + 15787	0.9995
Cortisone	y = 14872x + 13135	0.9991
Betamethasone	y = 14501x + 15139	0.9994
Dexamethasone	y = 19844x + 18502	0.9993

Table 3: Correlation coefficient, LOD, and LOQ results for corticosteroids by HPLC method

Dosage form	Corticosteroids	Correlation coefficient, R^2	LOD, mg/mL	LOQ, mg/mL
Tablet 5-25ppm	Triamcinolone	0.9722	0.57	0.90
	Prednisone	0.9927	0.54	1.14
	Prednisolone	0.9857	0.41	0.96
	Cortisone	0.9929	0.47	1.03
	Betamethasone	0.9916	0.37	0.89
	Dexamethasone	0.9810	0.42	1.02
Capsule 5-25ppm	Triamcinolone	0.9991	0.60	1.08
	Prednisone	0.9992	0.73	1.65
	Prednisolone	0.9991	0.64	1.57
	Cortisone	0.9997	0.70	1.68
	Betamethasone	0.9993	0.62	1.59
	Dexamethasone	0.9994	0.68	1.74

Table 4: Intermediate and recovery results for corticosteroids by HPLC method

Dosage form	Corticosteroids (10 mg/mL)	Recovery (%)	Intermediate precision (mg/mL) (n=6)				
			Day 1	Day 2	Avg	SD	%CV
Tablet	Triamcinolone	119.50	11.90	12.00	11.95	0.07	0.59
	Prednisone	98.35	9.97	9.70	9.83	0.19	1.94
	Prednisolone	95.65	9.45	9.68	9.56	0.16	1.70
	Cortisone	102.00	10.10	10.30	10.20	0.14	1.38
	Betamethasone	100.10	9.90	10.12	10.01	0.15	1.55
	Dexamethasone	112.80	11.12	11.44	11.28	0.22	2.00
Capsule	Triamcinolone	88.20	8.70	8.94	8.82	0.16	1.92
	Prednisone	96.60	9.55	9.77	9.66	0.15	1.61
	Prednisolone	96.35	9.50	9.77	9.63	0.19	1.98
	Cortisone	103.05	10.2	10.41	10.30	0.14	1.44
	Betamethasone	97.35	9.60	9.87	9.73	0.19	1.96
	Dexamethasone	100.80	10.00	10.16	10.08	0.11	1.12

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replicates for each concentration. It can be seen in table 3 that the values for the correlation coefficient values are over 0.90, which can be considered as showing good correlation between the concentration and peak area.

The precision parameter was carried out on the intraday study between two different days at 10 ppm concentration. The replicate for each corticosteroid was six and was analyzed separately on different days. It can be seen that the % CV or RSD for corticosteroids of the two matrices were below 2% (Table 4). According to G.A. Shabir (2003), the result on different days should have a statistical RSD ≤ 2%. Thus, it could be concluded that the result from this method is good and acceptable.

The recovery in table 4 was obtained by using equation 1. According to Sanco 2011 (SANCO/12495/2011 (2011)), the acceptance range for recovery was between 70 and 120%. The recovery from this study was in the range of 88.20 to 119.50%. It shows that the sample loss during the sample preparation was in the acceptable range.

CONCLUSION

In the present study, six corticosteroids were successfully separated and analyzed by using the developed and optimized HPLC method. The method was successfully validated and show acceptable result which fulfil the acceptance criteria for every validation parameter. The extraction or sample preparation method was also optimized on the sonication time and methanol water combination ratio for both studied matrix. The recovery data indicate the corticosteroid lost is within the allowed range. The present of corticosteroids in the matrix sample used in this study was not overlap or interfere by any peaks from the sample. This method could be used to evaluate the present of any studied corticosteroids either in tablet or capsule. Additional data to support the findings is by using the UV spectra for each corticosteroid (figure 3). However further confirmation should be carried out with mass detection which offer accurate result. Mass detection full fill the requirement for the registration of traditional and pharmaceutical product with Malaysia Ministry of Health (MOH) through NPRA which stated that the corticosteroids should not present in the product at all.

DECLARATIONS OF INTEREST

None

DECLARATION OF HONOUR

We declare in our honor that our results are not fake and made up.

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AI ASSISTANCE DISCLOSURE

The authors used [ChatGPT/GPT-5] to improve the clarity and readability of the manuscript. The authors carefully reviewed and edited the content to ensure accuracy and take full responsibility for the final text.

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