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A Facile HPLC-PDA Method for Simultaneous Determination of Paracetamol, Methyl Paraben, Sunset Yellow, and Carmosine in Oral Suspensions

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In the present study, a simple, fast, and accurate HPLC-PDA method was developed for the simultaneous determination of paracetamol (PAR), methylparaben (MP), sunset yellow (SSY) and carmoisine (CAR) in oral suspensions. The concentrations of colorants are less than with respect to those of active ingredient and this variation makes process of analysis troublesome. In the developed HPLC method, efficient chromatographic separation was achieved using reversed phase C18 column (4.6 mm x 150 mm x 5 µm particle size) and phosphate buffer solution (pH = 6.5)-acetonitrile mobile phase with a flow rate of 1.6 mL/min in the gradient mode. The eluents were monitored via a PDA detector at 300, 254 and 230 nm. The mean retention times of PAR, MP, SSY and CAR were found to be 2.15, 4.42, 1.58 and 3.81, respectively. The proposed method was validated in accordance with ICH guidelines and it was seen that the method met all requirements in terms of linearity, precision, accuracy, and selectivity. The developed method was successfully applied for simultaneous determination of the studied compounds in two commercial oral suspension samples.

Keywords: Paracetamol, methylparaben, sunset yellow, carmoisine HPLC-PDA, oral suspension.

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INTRODUCTION

Paracetamol (N-acetyl-4-aminophenol) is widely used in pediatric syrup-suspension formulations as a pain-reliever and fever-reducer. It is easily available alone without a prescription or in combination with other drugs. Some preservatives and colorants are commonly used in these pediatric pharmaceutical formulations to prevent microbial growth and to improve appearance and color (1). In general, sodium benzoate, potassium sorbate or parabens such as methyl paraben or propyl paraben are used as a preservant system in the liquid formulations (2). Methyl paraben (methyl 4-hydroxybenzoate) is the most frequently used due to its broad antimicrobial spectrum and the fact that it does not modify the physical properties of the final products like taste, smell or color (3). According to some research reports, parabens exhibit estrogenic activity and their extreme usage could lead to some detrimental effects such as breast cancers and oxidative DNA damage (4-6). Owing to these concerns, many countries have put a ban to limit the use of parabens (7). Hence, determinations of these preservatives in pharmaceuticals are vitally necessary for both quality assurance and consumer safety.

Although the allowable amounts of synthetic colorants which could have toxicity are reduced by human health reasons, many synthetic colorants in stead of natural colorants are still widely used due to their low price, high effectiveness, and excellent stability in foods and pharmaceuticals (8-10). Since synthetic colorants could provoke allergic reactions including urticaria, dermatitis, and asthma and may give rise to hyperactive behavior in children, the use of synthetic colorants in many countries is strictly regulated under existing food laws (11,12). Thus, determination of colorants in food samples and pharmaceutical products is important to control the amount of use permitted and to ensure quality control. Also, in order to colorize pharmaceuticals, the mixture of two or three of them is used to create a hue corresponding with selected natural color. So, simultaneous determination of these colorants becomes difficult as the number of components in the mixtures increases (13). Furthermore, since the quantity of active compound is commonly higher than additives, there is a need of accurate, efficient, and fast analytical method for simultaneous quantification of ingredients and excipients (14,15). In this respect, we focused on simultaneous high performance liquid chromatography (HPLC) determination of the single drug, paracetamol, along with colorants, sunset yellow and carmoisine, and preservative, methyl paraben, in pediatric oral suspension samples that belong to the same pharmaceutical company, in this study. Many analytical methods, either for single or combination with other analytes in various matrices, have been described in the literature for determination of paracetamol (16,17), methyl paraben (18,19), sunset yellow (20,21) and carmoisine (22,23) by HPLC in particular. However, no method describes quantification of this drug and color and preservative additives simultaneously. There are some HPLC methods developed in order to quantify different active compounds and additives in association, which are non labor-intensive, without extraction step and with short analysis time (2, 13, 24-27).

This study aims a fast, simple, and sensitive HPLC method for the simultaneous determination of paracetamol, methyl paraben, sunset yellow and carmoisine in pediatric oral suspensions. Validation parameters for the current method were also tested according to the requirements of ICH guidelines.

MATERIALS and METHODS

Chemicals and Reagents

Reference standards of paracetamol (PAR), methyl paraben (MP), sunset yellow (SSY) and carmoisine (CAR) were obtained as gifts from a local pharmaceutical company (all purities ≥ 99%). HPLC grade acetonitrile, methanol, orthophosphoric acid (H_3PO_4) and di-potassium hydrogen phosphate (K_2HPO_4) were purchased from Merck Chemicals (Germany). Ultrapure water was produced by a Milli-Q® Elix Water Purification System (Milford, MA, USA).

Instruments and Chromatographic Conditions

The HPLC system was a Shimadzu HPLC system LC-10AT VP equiped with a SIL-20AC autosampler and SPD-M10A VP photodiode array dedector (PDA). The chromatographic separations were performed on an Inertsil C18 column (4.6 mm x 150 mm x 5 µm particle size, GL Sciences, Japan). The mobile phase was made up of phosphate buffer (0.025 M, pH 6.5) and acetonitrile. Gradient elution conditions are given in Table 1. The flow rate of mobile phase was 1.6 mL/min at room temperature and injection volumes were 20 µL. The eluents were monitored in the range of 190 to 800 nm via a PDA dedector and the detections were carried out at 300 nm for PAR, at 254 nm for MP, at 230 nm for SSY and CAR. The run time was approximately 5 min and the total peak area was used for the quantification of each analyte.

Table 1. Gradient elution conditions for the separation of analytes.

Mobile Phase A	Mobile Phase B	Gradient Conditions
Acetonitrile	Phosphate buffer (0.025 M, pH 6.5)	87%B 2.0 min, 70%B 3.0 min

Preparation of Standard and Sample Solutions

Stock standard solutions of PAR (5000 µg/mL) and MP (500 µg/mL) were prepared in methanol by accurately weighting. Stock standard solutions of SSY and CAR (100 µg /mL) were prepared in Milli-Q water. All stock solutions were stored at 4 °c and further dilutions to obtain calibration and other validation studies solutions were made in Milli-Q water.

Two kinds of marketed liquid pharmaceutical formulations (suspension) comprising an aqueous solution of paracetamol were purchased from local pharmacy shop in Istanbul, Turkey. 2.5 mL of Suspension I containing 250 mg PAR, 4 mg MP and an unknown amount of SSY in 5 mL was

accurately transferred into an 100 mL measuring flask, sonicated in ultrapure water, and the volume was then made up to the mark with the same solvent. Similarly, 5.0 mL of Suspension II containing 120 mg PAR, 5 mg MP and an unknown amount of CAR in 5 mL was accurately transferred into a 50 mL measuring flask, sonicated and diluted to its volume with ultrapure water. All standard and sample solutions were filtered through 0.45 micron membrane filter.

Method Validation

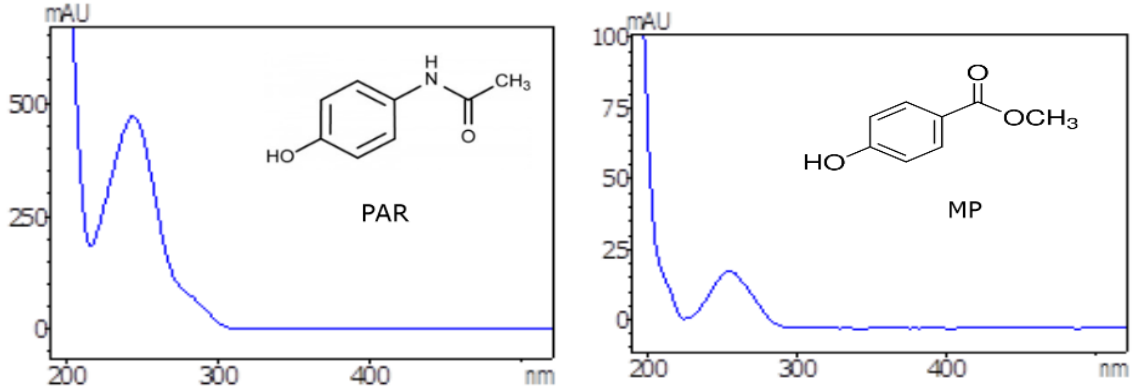
Method validation including selectivity, linearity, limit of detection and limit of quantification, precision and accuracy of the proposed method was performed according to ICH guidelines (28).

RESULTS and DISCUSSION

Method Development

In order to achieve good chromatographic separation of the studied compounds, different solvents (water, acetonitrile and methanol) and buffer solutions (acetic acid/acetate and phosphate), ideal mobile phase proportion, pH and flow rate were duly studied. Satisfactory results were achieved by 87:13% (v:v) of phosphate buffer (0.025 M) pH 6.5 : acetonitrile for first 2.0 min and then 70:30% (v:v) of the same mobile phase for 2.0-5.0 min at flow rate of 1.6 mL/min in the gradient mode.

As the quantity of active ingredient in these pharmaceutical formulations is compared to those of colorants in particular, it is seen that there is an imbalance between the analytes. Generally, the amount of the colorants is less than the active compound and excipients. This variation makes the simultaneous chromatographic analysis of them along with other ingredients difficult. This difficulty can be overcome by choosing the appropriate wavelength for determination. Absorption spectra of the studied compunds can be seen in Figure 1. Detection wavelength was chosen at 300 nm for PAR, at 254 nm for MP, at 230 nm for SSY and CAR, accordingly.



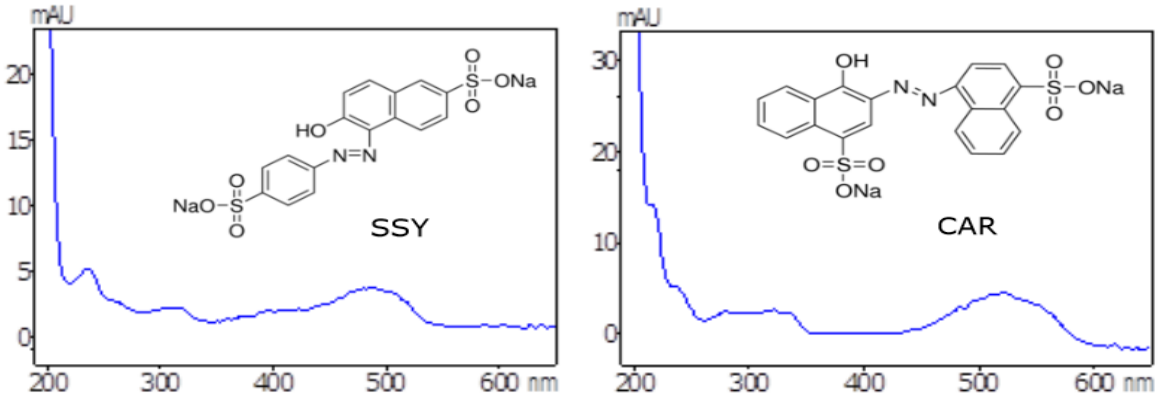
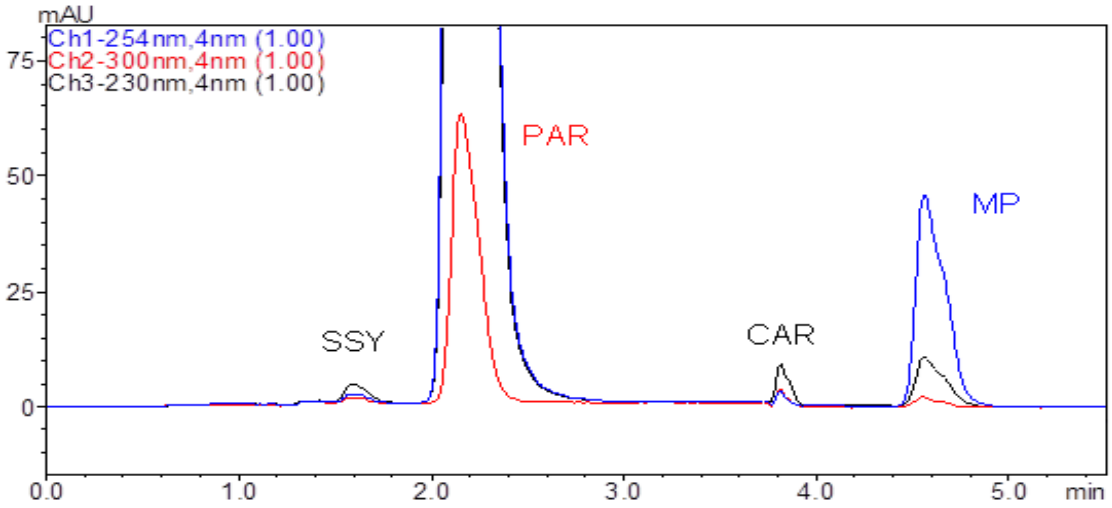
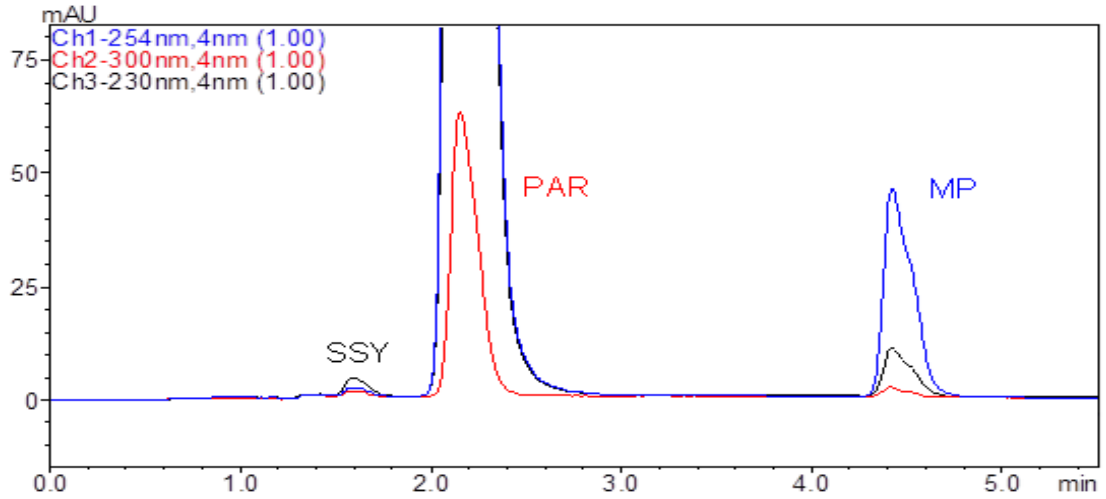


Figure 1. Absorption spectra of paracetamol (PAR), methyl paraben (MP), sunset yellow (SSY) and carmoisine (CAR) by PDA dedector.

Figure 2 shows the chromatogram obtained for the analytes in synthetic mixture and pharmaceutical formulations by the chromatographic conditions described above.



(a)



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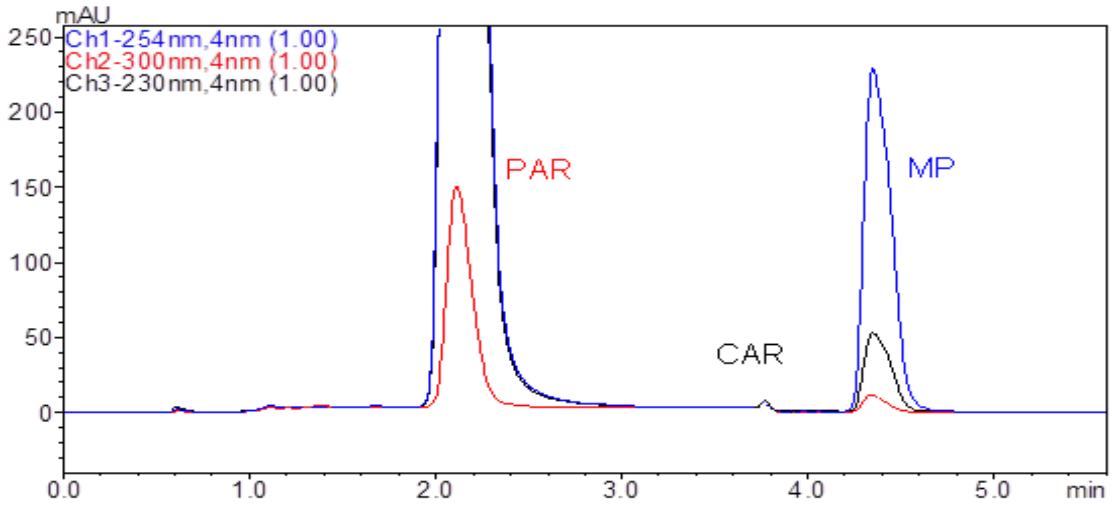


c)

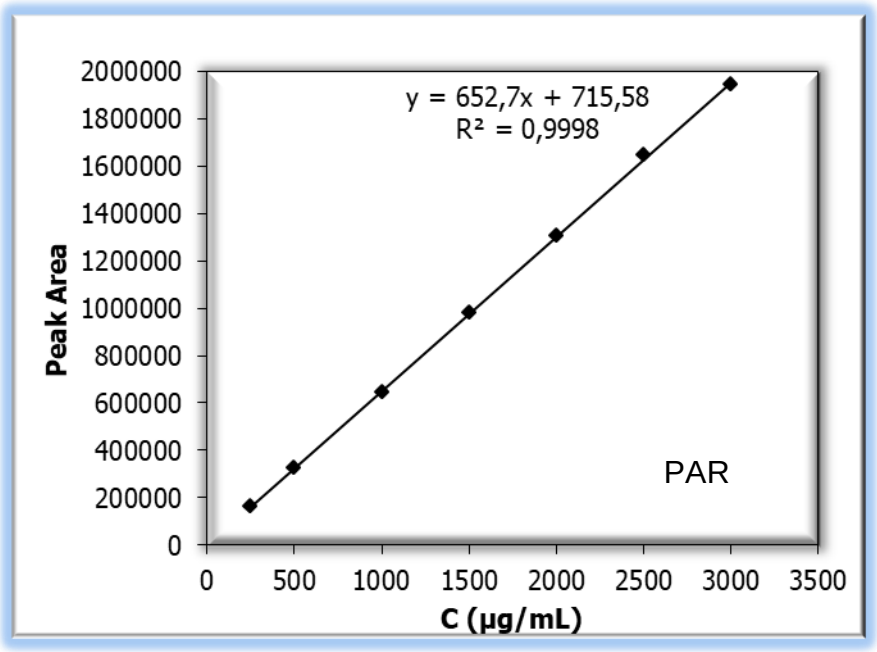
Figure 2. a) Chromatogram of standard mixture solution containing 4 µg/mL SSY, 1000 µg/mL PAR, 2 µg/mL CAR and 20 µg/mL MP. b) Chromatogram of oral Suspension I, c) Chromatogram of oral Suspension II.

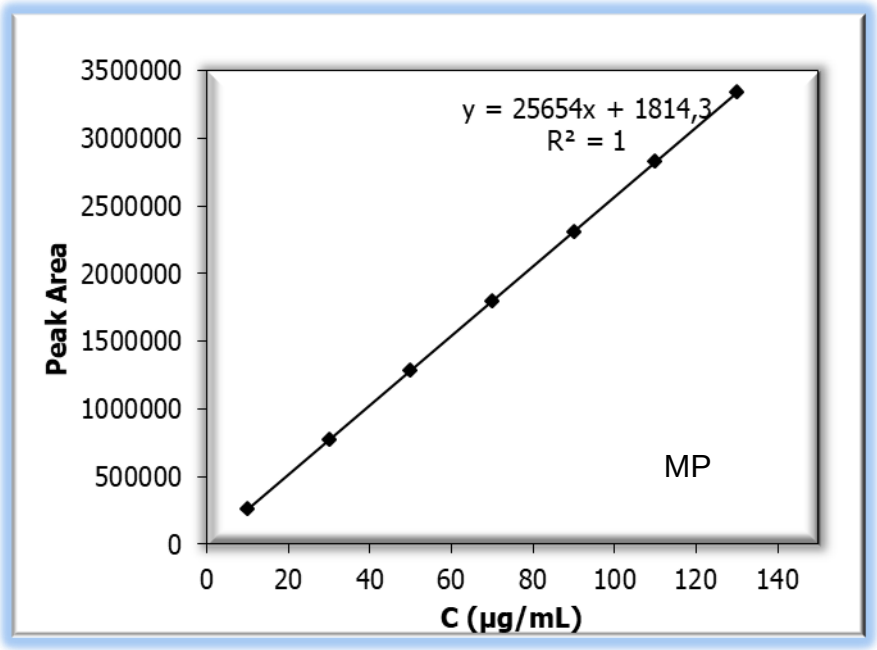
Method Validation

Selectivity: Selectivity was evaluated by the peak purity test using PDA detector. According to the results obtained, peak purity values were higher than 0.9990. Also no interferences were detected at retention times of the studied compounds in sample solutions, which shows that the developed method is selective (Figure 2).

Linearity: For linearity, seven different concentrations were chosen taking into account suspension contents. Hence, concentrations of the solutions were PAR 250 to 3000 µg/mL, MP 10 to 110 µg/mL, SSY 1 to 12 µg/mL and CAR 1 to 7 µg/mL. Each concentration of standard solutions was analyzed in triplicate and the mean values of peak areas were calculated and used

for the calibration graph. Calibration curves of the studied compounds obtained by the proposed method can be seen in Figure 3. The linear regression equations for PAR, MP, SSY and CAR were found to be $y = 652.7x - 715.58$, $y = 25654x + 1814.3$, $y = 10558x + 1532.5$ and $y = 15052x + 1742.6$, respectively. The regression cofficients (R^2) were found to be higher than 0.999, which indicates that the method has an acceptable degree of linearity.





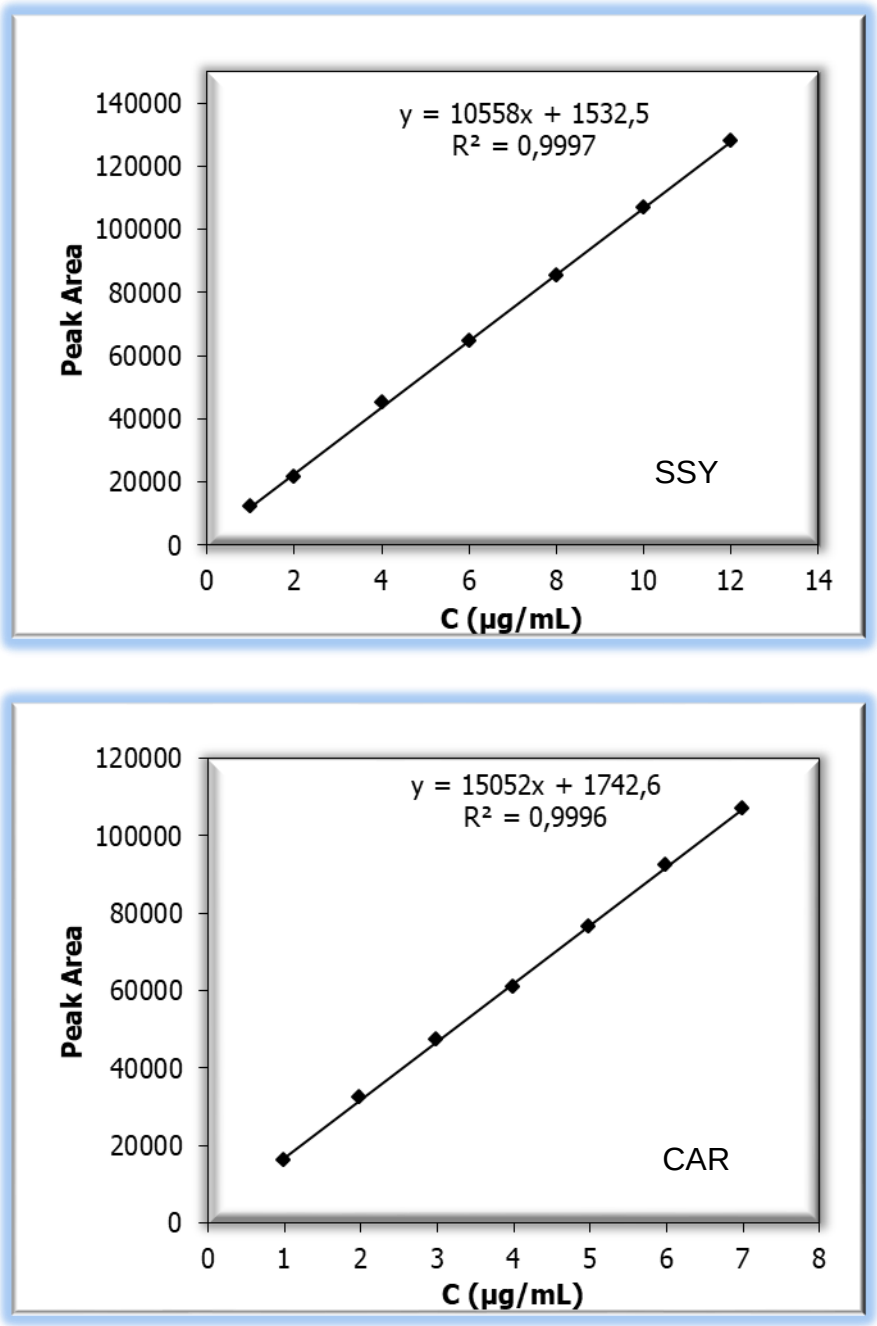


Figure 3. Calibration curves of PAR, MP, SSY and CAR.

Limits of Detection and Quantification (LOD and LOQ): Limits of detection (LOD) were calculated at a signal-to-noise ratio (S/N) of 3. Limits of quantification (LOQ) were calculated at a signal-to-noise ratio (S/N) of 10. The LOD was calculated to be 0.42, 0.20, 0.27 and 0.15 µg/mL and the LOQ was calculated to be 1.35, 0.65, 0.80 and 0.46 µg/mL for PAR, MP, SSY and CAR, respectively.

Precision: Precision of the method is assessed by the estimate of the relative standard deviation (RSD) with respect to both repeatability and intermediate precision. For repeatability, three different concentrations of standard solutions were analyzed in triplicate on the same day.

Intermediate precision (n=4) was performed on three different days. Acceptable RSD% values (<3.38% RSD) as shown in Table 2 were obtained.

Table 2. Repeatability and intermediate precision values of PAR, MP, SSY and CAR.

Compound	Actual Concentration (µg/mL)	Repeatability Mean ± %RSD (n =3) (µg/mL)	Intermediate precision Mean ± %RSD (n=4) (µg/mL)
PAR	1000	990.10 ± 1.52	993.75 ± 1.49
	1500	1479.33 ± 0.95	1487.00 ± 1.66
	2000	1992.67 ± 1.65	1991.75 ± 1.26
MP	20	20.47 ± 1.97	19.19 ± 2.45
	30	29.5 ± 1.70	29.83 ± 1.82
	40	39.6 ± 2.91	40.20 ± 2.96
SSY	2	2.07 ± 3.38	1.98 ± 2.80
	4	3.96 ± 3.26	4.08 ± 2.27
	6	5.97 ± 1.94	6.06 ± 2.27
CAR	1	0.98 ± 2.00	1.05 ± 2.52
	2	1.90 ± 2.80	1.95 ± 3.05
	3	3.10 ± 2.50	3.15 ± 2.65

Accuracy

Accuracy of the method was expressed as % recovery obtained by standard addition method at three different levels. Good recovery data for accuracy are displayed in Table 3. The recovery values obtained also imply that there is no matrix effect on the determination of analytes by the proposed method.

Table 3. Accuracy studies of PAR, MP, SSY and CAR.

Analytes	Sample Concentration (µg/mL)	Amount Added (µg/mL)	Amount Found (µg/mL) ± %RSD (n=5)	Mean Recovery (%)
PAR	1250	500	487.0 ± 1.03	97.40
		1000	1002.67 ± 0.75	100.27
		1500	1496 ± 0.68	99.73
MP	20	10	9.98 ± 0.76	99.80
		20	19.80 ± 1.35	99.00
		30	29.12 ± 2.00	97.07
SSY	1.75	2	1.97 ± 2.18	98.50
		4	3.95 ± 2.50	98.75
		6	6.05 ± 2.20	100.83
CAR	1.5	1	1.05 ± 2.44	105.00
		2	1.98 ± 1.96	99.00
		3	3.08 ± 2.15	102.67

Application of the Method to Commercial Oral Suspensions

After method optimization and validation, the developed method was successfully applied for the simultaneous determination of PAR, MP, SSY and CAR in oral suspension samples produced by the same pharmaceutical company. The quantitative results of the analysis are summarized in Table 4. Found values close to 100% demonstrate the applicability of the method for control of the liquid formulations in quality control laboratories. So these formulations containing the studied compounds can be analyzed with the same HPLC method in a short time.

Table 4. Analysis of marketed samples by the HPLC method.

	Ingredients	Labeled amount (mg/5 mL)	Amount found (mg/5 mL)	Recovery (%)
Commercial Oral Suspension I	PAR	250	250.6	100.24
	MP	4	4.05	101.32
	SSY	-	0.35	-
Commercial Oral Suspension II	PAR	120	121.2	101.0
	MP	5	5.11	102.29
	CAR	-	0.075	-

CONCLUSION

A novel, facile, rapid, and efficient reversed phase HPLC-PDA method without any extraction stage was developed for the simultaneous quantification of PAR, MP, SSY and CAR in oral suspensions which the ingredients are present in variable concentrations. The most important advantage of this method is to analyze PAR, MP, SSY and CAR at the same time as there is no method in the literature for simultaneous determination of these compounds. The developed method has a good resolution between all analytes with a short analysis time below 5 min. In addition, according to validation study results, this method is linear, precise, accurate, sensitive, and selective. So the proposed method can be used for routine analysis of these compounds in similar pharmaceutical products.

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that there is not an unethical situation.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ACKNOWLEDGEMENTSa

Declared none.

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