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Determination of Preservative Parabens in Oral and Injection Formulations by HPLC

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Two new HPLC-UV methods are described for determining methyl paraben and propyl paraben, methyl paraben sodium and propyl paraben sodium in oral and injection formulations. Separation of methyl paraben and propyl paraben, methyl paraben sodium and propyl paraben sodium by first method was effected by using an isocratic mobile phase of acetate buffer (pH = 4):methanol (18:82) on a 5 µm SGE C18 HQ 105 column (25 cm × 4.6 mm), the detector wavelength was set at 254 nm. Whereas the separation of methyl paraben and propyl paraben, methyl paraben sodium and propyl paraben sodium by second method was achieved by using mobile phase of acetate buffer (pH = 4):acetonitrile (20:80) on a 5µm SGE C18 HQ 105 column (25 cm × 4.6 mm), with UV detection at 254 nm. In both methods conditions, pump flow rate was 1 mL/min and sample injection volume 20 µL. Under these two methods conditions, separation of each two components was achieved in less than 6 min with a good resolution. Finally, these two HPLC methods are simple and quick for routine quantitative analysis of preservative parabens.

Key Words: Preservative, HPLC, Methylparaben, Propylparaben, Methylparaben sodium, Propylparaben sodium.

INTRODUCTION

Among many additives and preservatives have been widely used in food, drugs and cosmetic products to prevent their aging and decay[1]. They can be found in foods, beverages, pharmaceuticals and personal care products[2]. However, these preservatives may be harmful to consumers due to their tendency to induce allergic contact dermatitis. Parabens, esters of *p*-hydroxybenzoic acid (including methyl, ethyl, propyl, butyl, *etc.*) and their salts (Fig. 1) have been widely used as antimicrobial and antifungal agents in food, beverages, cosmetics and pharmaceuticals[3], due to their broad antimicrobial spectrum with good stability and non-volatility[4]. Therefore, the determination of these preservatives parabens in pharmaceutical products is important for both quality assurance and consumer safety. Many analytical procedures have been reported for the determination of methyl paraben and propyl paraben preservatives separately or in combination with other drugs by HPLC[5,6] and other techniques, such as UHPLC-tandem mass spectrometry[7], fluid extraction combined with liquid chromatography-mass spectrometry[8], spectrophotometric analysis[9], electrochemical analysis[10], flow injection analysis coupled to a monolithic column[11], fluid extraction combined with gas chromatography-mass spectrometry[12], NIR spectroscopy[13] and micellar electrokinetic chromatography. But there are no reports on the simultaneous determination of methyl paraben sodium and propyl paraben sodium.

The aim of the present study was to develop a speedily and sensitive HPLC conditions for the simultaneous determination of methyl paraben and propyl paraben or methyl paraben sodium and propyl paraben sodium.

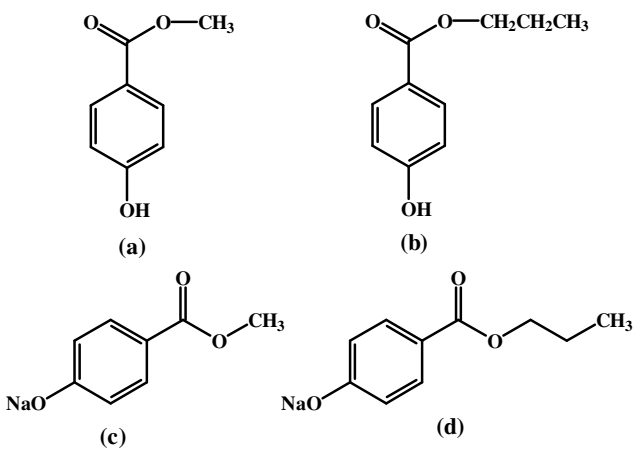


Fig. 1. Structure of the analytes: (a) methyl paraben, (b) propyl paraben, (c) methyl paraben sodium, (d) propyl paraben sodium

EXPERIMENTAL

The solvents and materials were used as analytical grade: water, ethanol (Merck), acetonitrile, methanol (Isolab) were HPLC grade and sodium acetate (Merck), glacial acetic acid

(Isolab), methyl paraben (purity 99.6 %) and propyl paraben (purity 99.22 %) (Clariant, UK), methyl paraben sodium (purity 99.85 %) and propyl paraben sodium (purity 99.7 %) (Salicylates and Chemicals, India).

HPLC analysis was performed on an YL 9100 HPLC System equipped with binary pump YL9111, vacuum degasser series YL9101, YL9130 column compartment and YL9120 UV/VIS detector. Chromatographic separations were obtained by using 5 µm SGE C18 HQ 105 column (25 cm × 4.6 mm). Ultrasonic bath (Daihan), analytical balance TE64 Sartorious sensitivity 0.01 mg, Germany digital pipettes (Isolab).

Standards stock solutions

Methyl paraben and propyl paraben solution: 1 mg/mL and 0.2 mg/mL of methyl paraben and propyl paraben was prepared respectively by dissolving 100.6 mg of methyl paraben and 20.2 mg of propyl paraben with 20 mL ethanol in 100 mL volumetric flask and diluted up to the volume with HPLC-grade water.

Methyl paraben sodium and propyl paraben sodium solution: 1 mg/mL and 0.2 mg/mL of methyl paraben sodium and propyl paraben sodium was prepared respectively by weighing 20.2 mg of propyl paraben sodium and 102.5 mg of methyl paraben sodium, in 100 mL volumetric flask and diluted to the volume by HPLC-grade water.

Sample preparation

Syrup: A 4 mL of syrup was transferred to a 100 mL volumetric flask, the volume was completed to 100 mL using HPLC-grade water and filtered through a 0.45 µm nylon syringe filter and degassed by ultrasonication and then the samples were injected in the chromatograph.

Ampoules: Containing five ampoules was transferred to a beaker then a 4 mL of it was transferred to a 100 mL volumetric flask, the volume was completed to 100 mL using HPLC-grade water, then filtered through a 0.45 µm nylon syringe filter and degassed by ultrasonication and then the samples were injected in the chromatograph.

Mobile phase: The mobile phase consisted of 18 % sodium acetate buffer and 82 % HPLC-grade methanol for the first method, 20 % sodium acetate buffer and 80 % HPLC-grade acetonitrile for the second method, (sodium acetate buffer was prepared by weighing 2.72 g sodium acetate and dissolving it in 1 L HPLC-grade water). Mobile phase pH was adjusted to 4 by glacial acetic acid. All mobile phase were filtered through a 0.45 µm membrane filter and degassed by ultrasonication.

Chromatographic conditions: Table-1 presents the first and the second chromatographic methods conditions, which were applied for simultaneous determination of methyl paraben and propyl paraben or methyl paraben sodium and propyl paraben sodium.

RESULTS AND DISCUSSION

Chromatograms of the standards solutions of mixture (methyl paraben and propyl paraben) at five different standard concentrations, which every concentration was injected five times under optimized in two methods conditions showed that methyl paraben was well separated from propyl paraben with good resolution (Fig. 2). Also chromatograms of the standards solutions of mixture (methyl paraben sodium and propyl paraben sodium) at five different standard concentrations which every one of it was injected five times under suggested chromatographic conditions in both methods gave good separation and resolution (Fig. 3). The separation of each two components in two methods was achieved in less than 6 min.

TABLE-1
FIRST AND SECOND CHROMATOGRAPHIC METHODS

Specification	First HPLC method	Second HPLC method
Column	SGE C18 HQ 105	SGE C18 HQ 105
Mobile phase	Acetate buffer (pH 4): methanol (18:82)	Acetate buffer (pH 4): acetonitrile (20:80)
Flow rate	1 mL/min	1 mL/min
Temperature	30 ºC	30 ºC
Detector	UV 254 nm	UV 254 nm
Injection volume	20 µL	20 µL

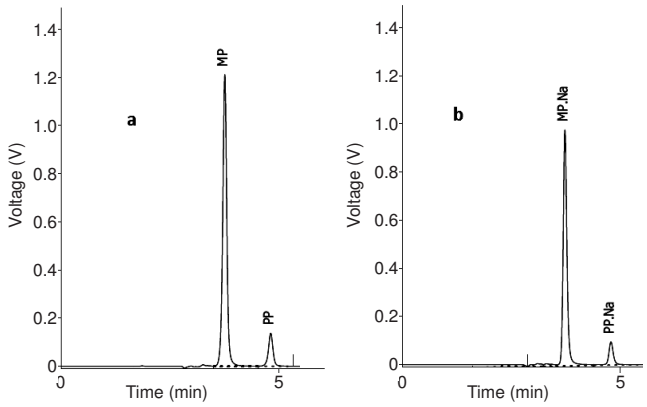


Fig. 2. Chromatogram of standards solutions showing separated peaks of: (a) methyl paraben (MP) and propyl paraben (PP); (b) methyl paraben sodium (MP.Na) and propyl paraben sodium (PP.Na), method (I)

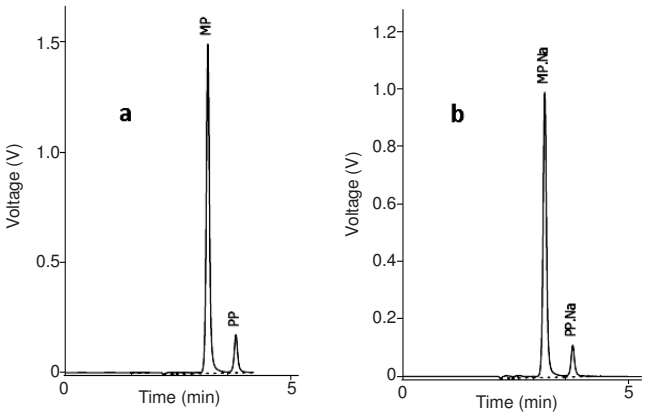


Fig. 3. Chromatogram of standards solutions showing separated peaks of: (a) methyl paraben (MP) and propyl paraben (PP); (b) methyl paraben sodium (MP.Na) and propyl paraben sodium (PP.Na), method (II)

Optimization of the HPLC conditions: The chromatographic conditions were optimized with respect to resolution and time of analysis.

pH effect of mobile phase: Using C18 RP column and mixture of organic solvent (methanol or acetonitrile) and buffers with different mobile phase pH values in range (pH =

2.5-6) was studied. The dependence of the peak area of (methyl paraben and propyl paraben), (methyl paraben sodium and propyl paraben sodium) against pH values was investigated.

The pH dependency of the peak area of the esters of *p*-hydroxy benzoic acid (methyl paraben and propyl paraben) and their salts by using (methanol or acetonitrile) and acetate buffer as mobile phase at constant mobile phase component ratios and injected quantity exhibits curves with maximum threshold at the pH range between 3.5-4.5 for two mentioned conditions.

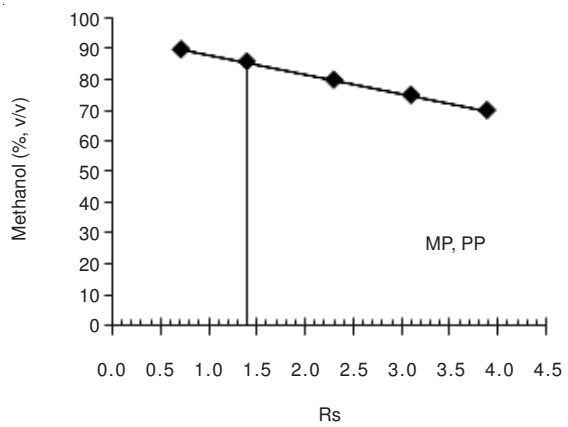
Increasing in the first part of the curves is due to lower protonation of the oxygen atom of the C=O group at higher pH values. At pH values higher than the maximums points of the parabens curves, partial process of deprotonation at the hydroxyl group takes place, which is the reason of decreasing of the absorbance at the specific wavelength.

By determining the best pH range of the preservatives application, we have to take into account the pH range of their stability. It is known that at pH above 8 a process of alkaline hydrolysis of parabens takes place, leading to *p*-hydroxy benzoic acid and the corresponding alcohol[14]. Therefore, pH ranges over 6.5 have to be avoided for parabens. In addition to silica solubility at higher pH than 8.

Peaks resolution: The peak resolution of (methyl paraben and propyl paraben) and (methyl paraben sodium and propyl paraben sodium) peaks was identified, by changing the ratios of mobile phase components in both methods at constant mobile phase pH = 4. It was concluded that each two peaks was completely separated at the mobile phase ratio, acetate buffer:methanol (14:86) in first method, but in second method the mobile phase ratio was acetate buffer:acetonitrile (17:83). Both mobile phase ratios were obtained at completed separation where peak resolution (Rs) = 1.4, as seen in (Figs. 4 and 5). Practically, It was found that the best ratio of the mobile phase components where Rs > 1.4, with obtaining undeformed, symmetrical and fine peaks was presented in Table-1.

Methods validation

Linearity: System linearity was determined by analysis of five replicates of five standards concentrations of (methyl paraben and propyl paraben), (methyl paraben sodium and propyl paraben sodium) in both methods (I, II), as it is seen in Figs. 6 and 7 (Table-2).



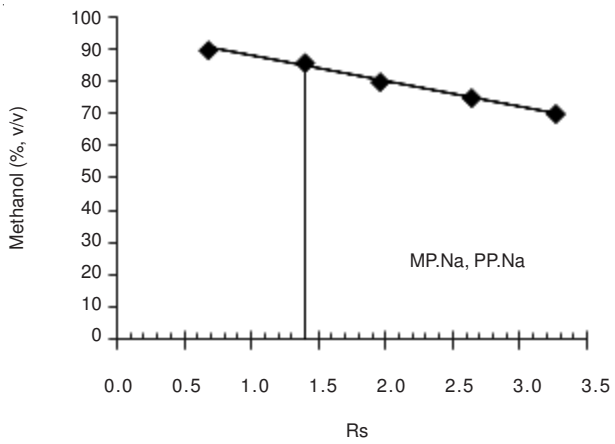
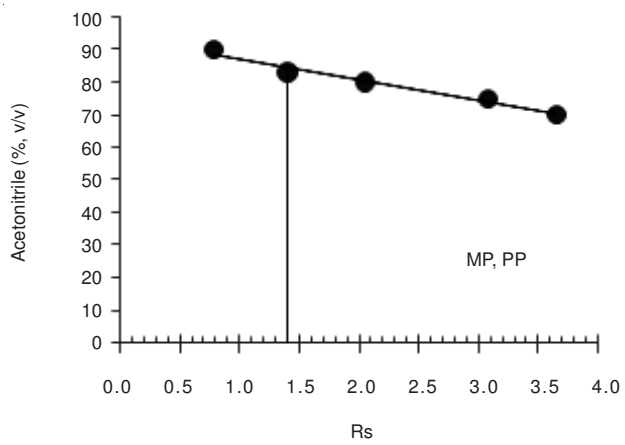


Fig. 4. Peaks resolution Rs for [methyl paraben (MP) and propyl paraben (PP)], [methyl paraben sodium (MP.Na) and propyl paraben sodium (PP.Na)], method (I)



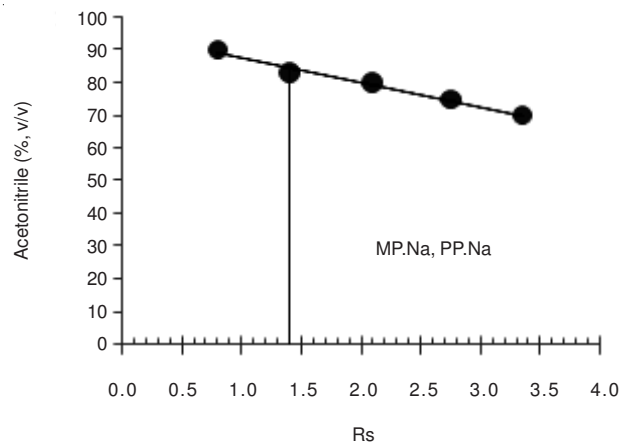


Fig. 5. Peaks resolution Rs for [methyl paraben (MP) and propyl paraben (PP)], (methyl paraben sodium (MP.Na) and propyl paraben sodium (PP.Na)], method (II)

Accuracy/recovery: The accuracy of the method was checked by evaluating the experimental concentration of the solutions prepared for the linearity test versus the nominal concentration. Good recovery of (methyl paraben and propyl paraben), (methyl paraben sodium and propyl paraben sodium) by two methods was observed as shown in Table-2.

TABLE-2
SOME OF THE VALIDATION PARAMETERS FOR PRESERVATIVES ASSAY

	Component	Linearity (mg/mL)	Regression equation	Correlation coefficient	Recovery average (%)	Precision (% RSD) System Precision (n = 5)	Limit of quantification (µg/mL)	Limit of detection (µg/mL)
First method	Methyl paraben	0.020-0.20	y = 12.12x + 0.056	0.999	100.07	0.87	4.50	1.50
	Propyl paraben	0.004-0.04	y = 10.84x + 0.012	0.999	100.33	0.90	1.70	0.50
	Methyl paraben sodium	0.020-0.20	y = 11.60x – 0.006	0.999	100.29	1.32	6.50	2.20
	Propyl paraben sodium	0.004-0.04	y = 8.013x + 0.002	0.999	99.99	0.87	2.02	0.67
Second method	Methyl paraben	0.010-0.14	y = 12.52x + 0.032	0.999	100.22	0.57	1.90	0.60
	Propyl paraben	0.002-0.03	y = 10.71x + 0.012	0.999	100.09	0.84	0.56	0.18
	Methyl paraben sodium	0.010-0.14	y = 10.01x + 0.031	0.999	100.51	0.66	2.40	0.80
	Propyl paraben sodium	0.002-0.03	y = 7.482x + 0.006	0.999	100.06	0.73	0.66	0.22

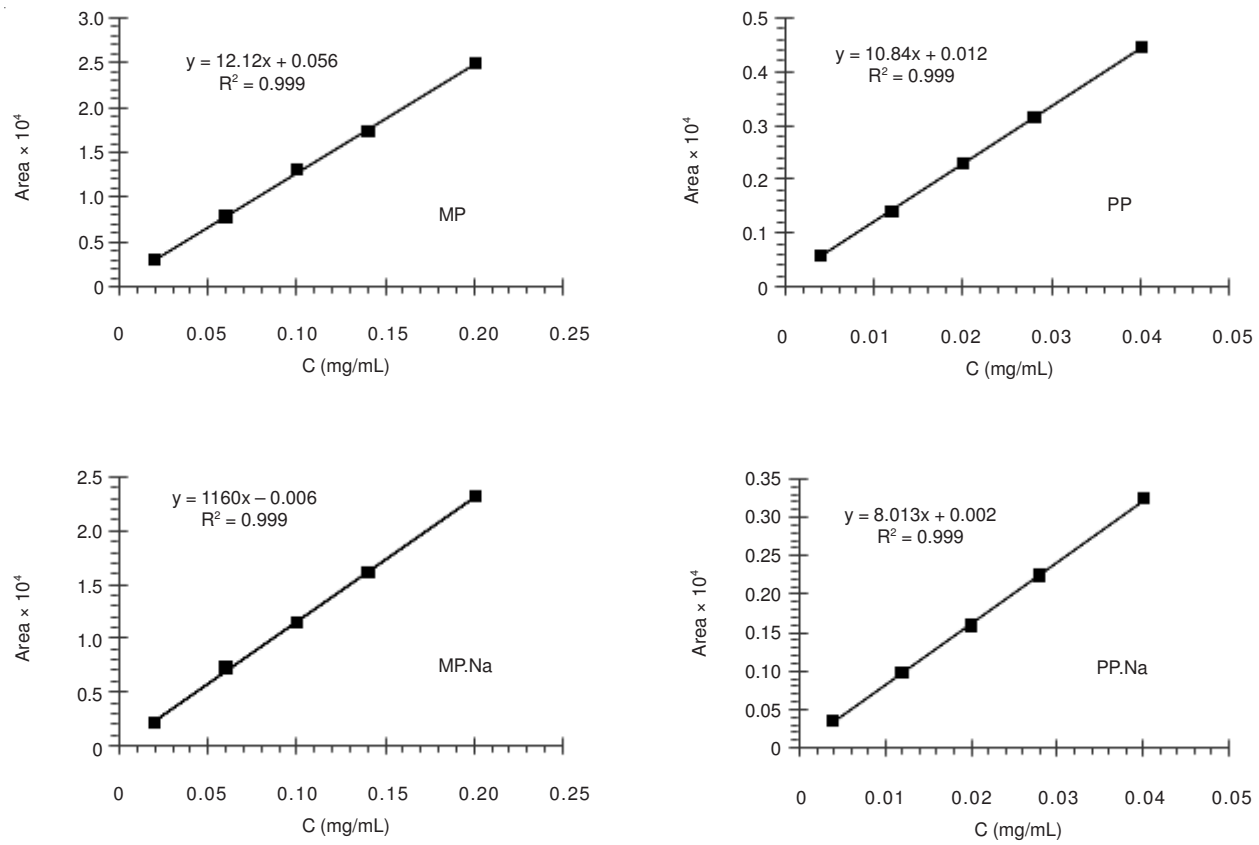


Fig. 6. Linearity graphs of [methyl paraben (MP) and propyl paraben (PP)], [methyl paraben sodium (MP.Na) and propyl paraben sodium (PP.Na)], method (I)

Precision

System precision: Five replicates (n = 5) of a standard mixture solution (0.1 mg/mL methyl paraben and 0.02 mg/mL propyl paraben), (0.1 mg/mL methyl paraben sodium and 0.02 mg/mL propyl paraben sodium) were analyzed to assess system precision. The RSD of peak area response (Table-2) showed the satisfactory repeatability of the system (< 2 %).

Method precision: Five replicates (n = 5) of sample solutions were analyzed in the same day to determine method precision. The low RSD (< 2 %) showed the suitability of the method for the determination of (methyl paraben and propyl paraben), (methyl paraben sodium and propyl paraben sodium) in oral and injection formulation, the method precision was summarized in Table-3.

Limit of detection (LOD) and limit of quantification (LOQ): The limit of detection and limit of quantification were obtained from the calibration curves. The limit of detection and limit of quantification were calculated based on the standard deviation of the intercept (SD = δ) and the slope (S) of the calibration curves using the formulae 3.3 δ/S and 10 δ/S respectively. The limit of detection and limit of quantification concentrations were reported in Table-2.

Specificity: It was determined (methyl paraben and propyl paraben), (methyl paraben sodium and propyl paraben sodium) in both methods in all samples without any interference. Peaks were completely separated with good resolution and specificity. Confirming the specificity of the method; the peaks of (methyl paraben and propyl paraben), (methyl paraben sodium and propyl paraben sodium) in the samples

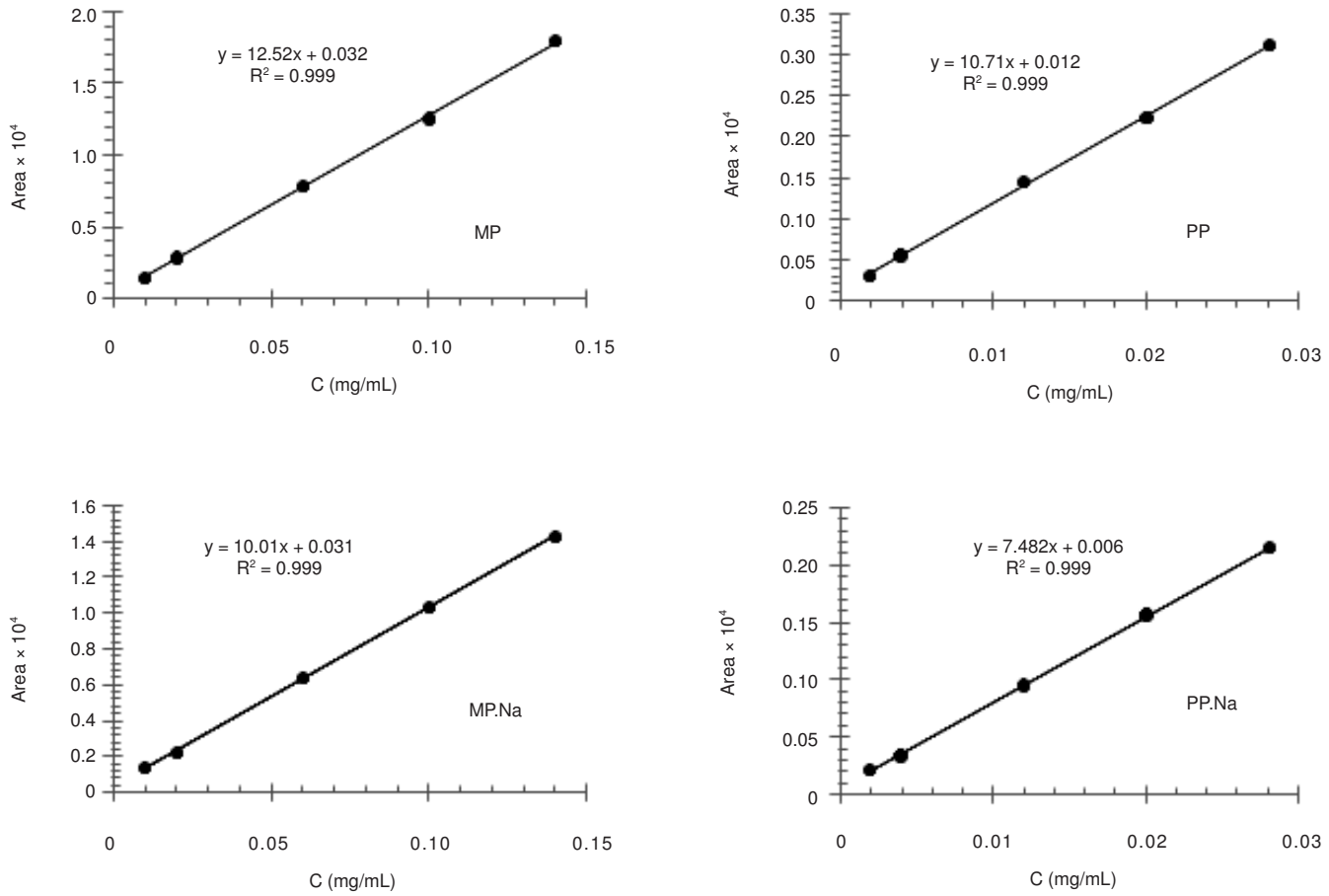


Fig. 7. Linearity graphs of [methyl paraben (MP) and propyl paraben (PP)], [methyl paraben sodium (MP.Na) and propyl paraben sodium (PP.Na)], method (II)

TABLE-3
METHYL PARABEN, PROPYL PARABEN AND THEIR SALTS CONTENTS IN ORAL AND INJECTION FORMULATIONS PRODUCTS

Trade name	Company	Component	Method (I)		Method (II)	
			$\overline{X}(mg/mL)_{n=5}$	RSD (%)	$\overline{X}(mg/mL)_{n=5}$	RSD (%)
Amrivite-F (Injection)	Amrit	Methyl paraben sodium	1.25	0.93	1.12	0.69
		Propyl paraben sodium	0.26	1.88	0.27	1.43
Amrivite (Injection)	Amrit	Methyl paraben sodium	1.34	1.45	1.07	1.02
		Propyl paraben sodium	0.25	1.82	0.26	1.80
Dexamrit (Injection)	Amrit	Methyl paraben sodium	1.39	1.68	1.25	0.87
		Propyl paraben sodium	0.21	1.86	0.19	1.88
Stigmin (Injection)	Amrit	Methyl paraben sodium	1.33	0.73	1.06	1.42
		Propyl paraben sodium	0.16	1.76	0.22	1.95
Ketotiphen (Syrup)	Amrit	Methyl paraben	1.23	0.68	1.44	1.02
		Propyl paraben	0.21	1.03	0.18	1.51
Dexamrit (Syrup)	Amrit	Methyl paraben	0.79	1.21	1.09	1.26
		Propyl paraben	0.17	1.52	0.18	1.76
Salbutamol (Syrup)	Amrit	Methyl paraben	1.34	1.30	1.49	1.37
		Propyl paraben	0.21	1.73	0.17	1.70
Acephyllin (Syrup)	Amrit	Methyl paraben	1.09	1.37	1.24	0.95
		Propyl paraben	0.18	1.52	0.19	1.79
Algiscon (Oral suspension)	Delta	Methyl paraben	1.16	1.13	1.23	1.05
		Propyl paraben	0.27	1.45	0.28	1.34
Pectomex (Syrup)	Razi	Methyl paraben	1.29	1.01	1.42	0.95
		Propyl paraben	0.37	1.16	0.43	1.22
Barkacine (Drops)	Barakat	Methyl paraben	1.18	0.99	1.26	1.86
		Propyl paraben	0.52	1.41	0.56	1.89

were identified by comparing the retention time with that of the standards.

Application of the assay: The applicability of two HPLC-UV methods was investigated for pharmaceutical preparations (syrup, ampoule). In all the preparations, the amount of (methyl paraben and propyl paraben), (methyl paraben sodium and propyl paraben sodium) was obtained by direct measurement using the standard calibration curve. The total analysis time was less than 6 min with good resolution, good peak shapes and minimal tailing. Four pharmaceutical formulations containing (methyl paraben sodium and propyl paraben sodium) and seven pharmaceutical formulations containing (methyl paraben and propyl paraben) were analyzed. The results obtained by the proposed methods are in satisfactory agreement. The analytical results are summarized in Table-3.

Conclusion

Proposed HPLC methods are direct, specific, rapid and precise for simultaneous determination of (methyl paraben and propyl paraben), (methyl paraben sodium and propyl paraben sodium) in oral and injection formulations. It was found that the second method is more sensitive and faster than the first one.

The described two methods are suitable for routine analysis and quality control of oral and injection containing these two preservative ingredients, either alone or in combination.

It was found that there is very small difference of mobile phase ratio, necessary to separate methyl paraben and propyl paraben or their salts in both methods.

In samples contain (methyl paraben and propyl paraben) or their sodium salts, the added amounts of those preservative were in allowable safety limits and did not exceed the authorized limits.

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