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DEVELOPMENT AND VALIDATION OF AN RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF POTASSIUM SORBATE AND SODIUM BENZOATE FROM HERBAL SYRUP FORMULATION

[1]*Unmesh Keshwar, [2]Pranali Ingole, [3]Sharad Dhurde and [4]B. K. Shrikhande

[1]ADL Manager, Siddhayu Ayurvedic Research Foundation Pvt. Ltd, Nagpur.
[2]Research Executive, Siddhayu Ayurvedic Research Foundation Pvt. Ltd, Nagpur.
[3]Manager, Siddhayu Ayurvedic Research Foundation Pvt. Ltd, Nagpur.
[4]Director, Siddhayu Ayurvedic Research Foundation Pvt. Ltd, Nagpur.

***Corresponding Author: Unmesh Keshwar**

ADL Manager, Siddhayu Ayurvedic Research Foundation Pvt. Ltd, Nagpur.

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ABSTRACT

Background: Most of the herbal formulations contain synthetic preservatives like potassium sorbate (PS) and sodium benzoate (SB) for preventing the microbiological contamination. Identification and quantification of these preservative is mandatory requirement with respect to quality and consumers safety. Hence Siddhayu Ayurvedic Research Foundation Pvt. Ltd. has developed and validated the method. **Objective:** Objective of the present study was to develop the method with good resolution for simultaneous estimation of potassium sorbate and sodium benzoate using RP-HPLC method from herbal syrup formulation and validate it. **Methods:** In this RP-HPLC method, an analytical C18 column was used to achieve simultaneous chromatographic separation by using Buffer solution: Acetonitrile: Tetrahydrofuran (80:10:10 v/v/v) as mobile phase at flow rate of 1.5 ml/min. Quantification was carried out using a photodiode array detector at 232 nm. This developed method was also validated according to International Conference on Harmonization guidelines. **Results:** The retention time of PS and SB was 10.93 and 13.95 minutes respectively with good resolution. The estimated amount of PS and SB was found to be 0.0993 % and 0.2014 % in the herbal syrup formulation. All the test results of the evaluated validation parameters were found well within the limits. **Conclusion:** The developed method is able to separate the potassium sorbate and sodium benzoate simultaneously with good resolution. Hence, the present analytical method is applicable for simultaneous determination of PS and SB in herbal syrup formulation. Also results obtained in validation parameters indicate the reliability of the developed simultaneous HPLC method.

KEYWORDS: Potassium sorbate, Sodium benzoate, herbal syrup formulation, RP-HPLC.

INTRODUCTION

Herbal formulations are composed of active ingredients sourced from natural origins. Due to their natural composition, these materials are prone to contaminate with the microbial growth. Most of the herbal formulation manufacturers are using synthetic preservatives to control this microbiological contamination. Sodium benzoate (SB) and Potassium sorbate (PS) are the most common preservatives which are used in the food products as well as herbal formulations.[1,2]

Sodium benzoate, derived from the substitution of a proton with a sodium ion in benzoic acid's carboxy group, is a preservative commonly used in foods, pharmaceuticals and cosmetics. It has a role as an antimicrobial food preservative, a drug allergen, an EC 1.13.11.33 (arachidonate 15-lipoxygenase) inhibitor, an EC 3.1.1.3 (triacylglycerol lipase) inhibitor, an algal metabolite, a human xenobiotic metabolite and a plant metabolite.[3] Similarly, potassium sorbate, a potassium salt featuring sorbate as the counter ion, is employed primarily as an antimicrobial food preservative. It functions as a mold and yeast inhibitor, serving as a fungistatic agent in various food products.[4]

Estimation of both these preservatives potassium sorbate and sodium benzoate in herbal formulations is necessary with respect to quality and consumer safety aspects, as their uses are associated with the risk of many side effects and pose health risks for consumers at higher concentrations.[5-8] The World Health Organization (WHO has also emphasized the importance of monitoring the stability of preservatives throughout the shelf-life of herbal formulations.[9]

While various methods for estimating potassium sorbate and sodium benzoate and in different food products have been reported, a lack of suitable chromatographic separation and resolution for simultaneous identification and quantification from multicomponent herbal formulations by High-Performance Liquid Chromatography (HPLC) has been noted.[10-16]

Acknowledging these challenges, Siddhayu Ayurvedic Research Foundation Pvt. Ltd. has developed and validated a Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) method for the simultaneous estimation of potassium sorbate and sodium benzoate in herbal formulations. This study aims to present a method suitable for routine use in Quality Control Laboratories, validated according to International Conference on Harmonization (ICH) guidelines Q2 (R1).[17]

MATERIALS AND METHODS

Instrumentation

Instrumentation details are mentioned in below Table 1.

Table 1: Instrumentation details.

Sr. No.	Instruments	Specification
1	HPLC instrument	Waters Arc HPLC
2	Detector	Photo diode Array Detector (2998 PDA)
3	wavelength range	210 nm to 800 nm
4	Software	Empower
5	Balance	Mettler Toledo
6	pH meter	Toshnival
7	Sonicator	Tempo

REAGENTS AND MATERIALS

All chemicals used throughout this work were of analytical grade or HPLC grade purchased from Merck Chemicals, India. Water used for the analytical work is of Type 1 purified water. Reference standard Potassium sorbate (99.7 %) and Sodium benzoate (100.0 %), were purchased from Sigma Aldrich, India.

PREPARATION OF SOLUTIONS

Preparation of Standard-stock Solutions

The standard solution were prepared by weighing 5.0 mg of Potassium Sorbate (PS) and 10.0 mg Sodium Benzoate (SB) reference standard and then transferred to 100 ml volumetric flask and volume was adjusted with diluent (Acetonitrile and water 50:50) to obtain concentration 50 µg/ml (50 ppm) for PS and 100 µg/ml (100 ppm) for SB.

Preparation of Working-standard Solution

From the standard stock solution 5 ml solution was pipette out and diluted with diluent (Acetonitrile and water 50:50) up to 50 ml in volumetric flask to obtain concentration of 5 µg/ml (5 ppm) for PS and 10 µg/ml (10 ppm) for SB. This solution was used as working standard solution.

Preparation of test solution (Herbal Syrup Formulation)

Accurately 5000 mg of syrup sample was weighed in 100 ml of volumetric flask. About 80 ml of diluent (Acetonitrile and water 50:50) was added and solution was sonicated on ultrasonic water bath for 15 min at room temperature. The solution was allowed to cool at room temperature. Further, volume was made up to the mark with diluent. From the resulting solution 5.0 ml was diluted upto 50 ml with diluent. The solution was filtered through 0.45 µ syringe filter and used as test solution.

Preparation of Buffer Solution

Buffer Solution was prepared by dissolving 2.7218 g of potassium dihydrogen phosphate in water (1000 ml), and pH was adjusted with ortho-phosphoric acid to 2.5.

Mobile Phase Preparation

Mobile phase was prepared by using Buffer solution, Acetonitrile and Tetrahydrofuran in ratio of 80:10:10 v/v/v.

Method Development

Various mobile phases were tried to obtain the simultaneous elution of both the components PS and SB with good resolution and sharp peak. After various trials, the mobile phase containing Buffer solution, Acetonitrile and Tetrahydrofuran in ratio of 80:10:10 v/v/v meets the criteria and finalised for the analysis. For the UV spectra, solutions of Standard PS and SB were scan over a range of 200 nm to 400 nm in UV region. After scanning obtained spectra were overlaid and maximum absorption wavelength (isosbestic point) was selected at 232 nm.

Chromatographic Conditions

The details of optimized parameters of chromatographic conditions for estimation of PS and SB are mentioned in Table 2.

Table 2: Optimized chromatographic conditions.

Sr. No.	Parameter	Optimized conditions
1	Mobile Phase	Buffer solution (pH 2.5), Acetonitrile and Tetrahydrofuran
2	Ratio	(80:10:10 v/v/v)
3	Column	C18 (5 µm 4.6 x 250 mm)
4	Flow rate	1.5 ml/min
5	Wavelength	232 nm

6	Injection Volume	20 µl
7	Column Temperature	25°C
8	Sample Temperature	25°C

Assay of PS and SB in Herbal Syrup Formulation
Herbal Syrup Formulation was analysed for the assay of PS and SB as per the developed and optimised method. Sample and standard solution were injected under the same conditions. Sample solution was analysed in triplicate.

Analytical Method Validation
The developed RP-HPLC method was validated for the parameters like system suitability specificity, linearity, accuracy, range, precision, and robustness as per the International Conference on Harmonization (ICH) guidelines Q2 (R1). The details of the analytical method validation are as follows.

System suitability
System suitability was performed on standard PS and SB having concentration 5 ppm and 10 ppm respectively. Retention time, resolution, peak shape, theoretical plates, tailing factor and % RSD were ascertained for the suitability of the instrument for getting accurate and precise results.

Specificity
The Specificity was proved by chromatographic comparison of blank, Placebo solution, standard solutions and the test solution. No interference in the peak observed from blank and placebo solution at the Retention time of Reference standard solutions confirms the specificity.

Linearity
Linearity for PS and SB standard solution was performed by evaluating minimum five concentrations. The slope, intercept and correlation coefficient should be reported by plotting the linearity graph of peak area against concentration of standards.

Accuracy
The accuracy was carried out by the standard addition technique by spiking the analyte into the matrix of the sample (placebo). Accuracy was assessed by minimum 9 determinations over a minimum of 3 concentration levels (3 Concentration / 3 replicate) covering the specified range by adding known amount of actives in placebo solution. These analysed samples and obtained results were compared with expected results.

Range
The range was derived from the linearity studies and accuracy. Range of an analytical method is the interval between the upper and lower concentration of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity.

Precision
Precision was evaluated in terms of system precision, method precision and intermediate precision. The system precision was determined by six measurements of mix standard solution of PS and SB containing each analyte at 100% of target concentration on the same day. The method precision includes repeatability and intermediate precision. They were determined by six measurements of sample solution containing each analyte at approximately 100 % of target concentration on the same day and on two different days, respectively. Overall RSD of 12 samples was calculated by taking precision and intermediate precision into consideration.

Robustness
The influence of slightly changed parameters of the chromatographic conditions was tested according to ICH guidelines to demonstrate sufficient robustness of the method. For assessment of the robustness of developed analytical method parameters like flow rate, pH of mobile phase buffer and mobile phase composition were deliberately changed.

RESULTS AND DISCUSSION
The RP-HPLC method was successfully developed for the simultaneous estimation of PS and SB having retention time 10.93 and 13.95 minutes respectively with good resolution and sharp peak.

Assay Results
Assay of PS and SB as per the developed and optimised method was performed in triplicate and results were incorporated in the Table 3.

Table 3: Results of Assay.

Sample ID	PS		SB	
	Assay (%)	Mean	Assay (%)	Mean
Test Sample-1	0.0998	0.0993	0.2028	0.2014
Test Sample-2	0.0993		0.2017	
Test Sample-3	0.0988		0.1997	

Validation Results
This developed method was also validated as per the as per the International Conference on Harmonization (ICH) guidelines of which results are mentioned and discussed below.

System suitability Assessment
Standard Solution of PS and SB as per developed method were prepared and injected into HPLC system. Retention time, resolution, peak shape, theoretical plates, tailing factor and % RSD were evaluated and results were tabulated in the following Table 4.

Table 4: Results of System suitability.

Sr. No.	Name of Analyte	Retention Time (RT)	Mean Peak Area	Theoretical Plates	Tailing Factor	% RSD	Resolution
1	PS	10.93	150377	5645	1.06	0.4	4.4
2	SB	13.95	424645	5514	1.04	0.2	

Specificity Assessment

Blank Solution, Placebo Solution, Standard Solution and Test Solution were injected and chromatograms were recorded. The Retention time was observed at 10.93 and 13.95 minutes for PS and SB respectively in Standard Solution. No peak interference was observed in blank and placebo at the retention time PS and SB. No peak purity flag was observed in Standard and Sample solution. The chromatograms are given below in "Fig. 1-4".

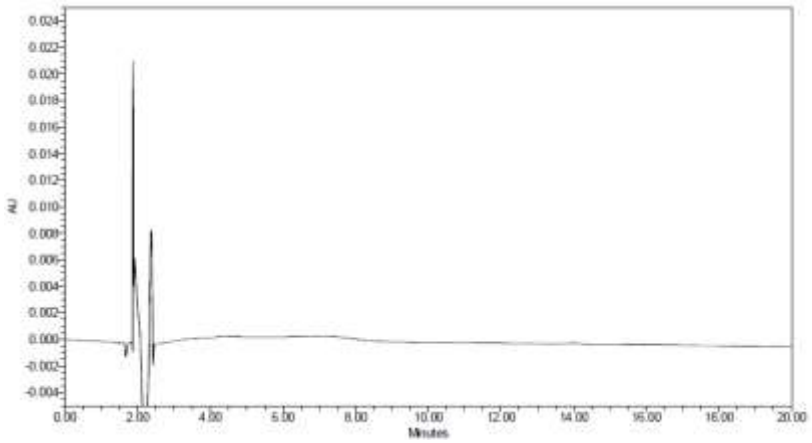

Figure 1: Chromatogram of Blank Solution.

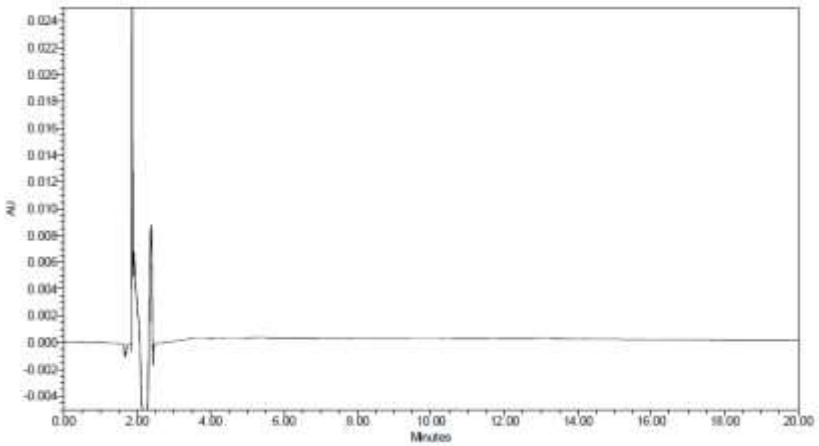

Figure 2: Chromatogram of Placebo Solution.

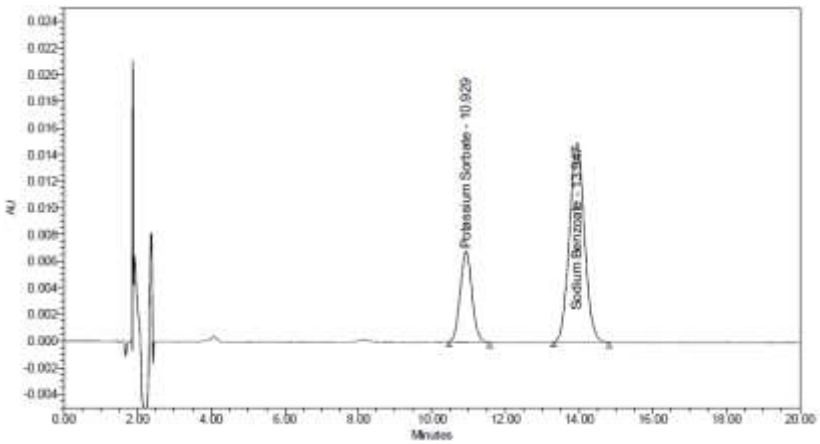

Figure 3: Chromatograms of Standard Solution.

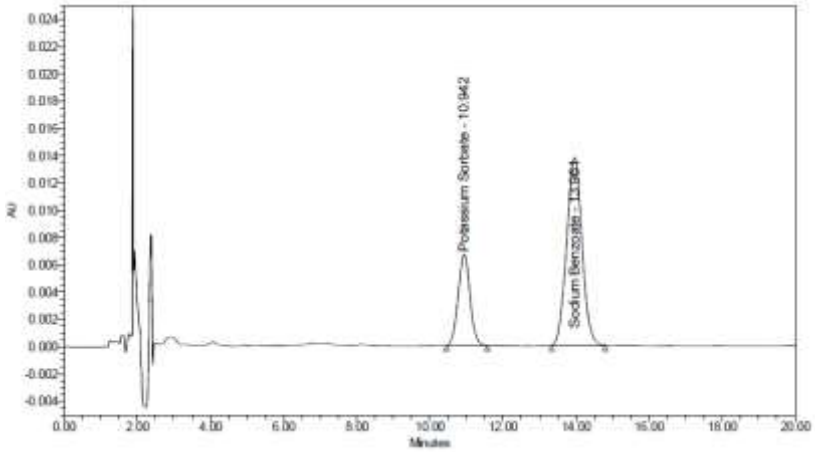

Figure 4: Chromatograms of Sample solution.

Linearity Assessment

The method gave linear response with concentrations of 2.5, 4.2, 5.0 6.0 and 7.5 ppm for PS and 5.0, 8.4, 10.0, 12.0 and 15.0 ppm for SB. Linearity curve was obtained by plotting a graph of peak area vs. concentration. All data were calculated and given in Table 5 and "Fig. 5, 6".

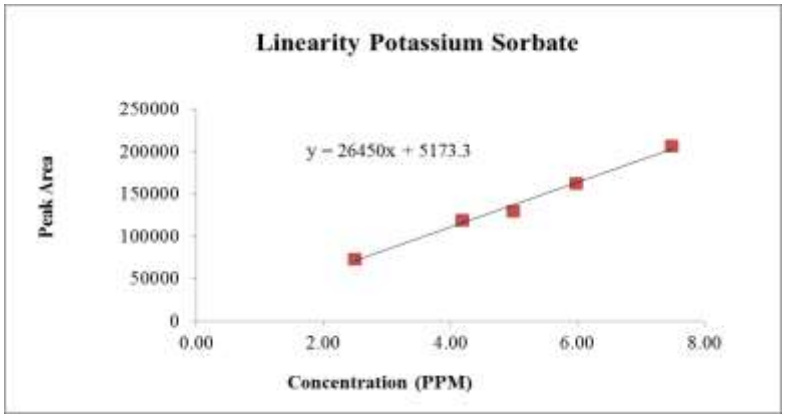

Figure 5: Linearity Graph of Potassium Sorbate.

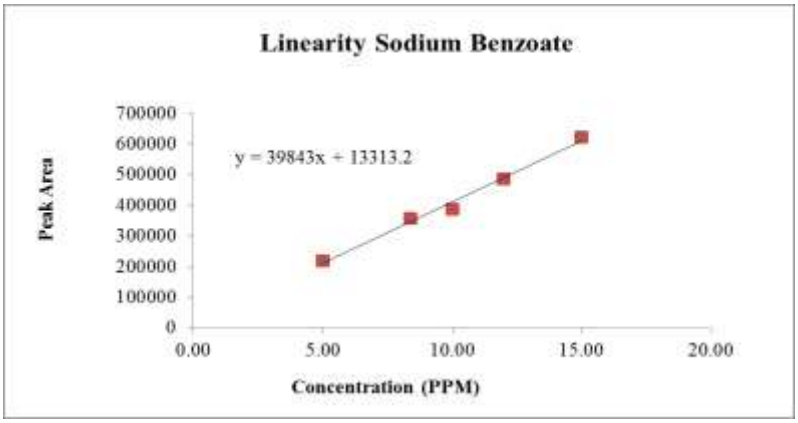

Figure 6: Linearity Graph of Sodium Benzoate.

Table 5: Results of Linearity.

Sr. No.	Parameter	Results	
		Potassium Sorbate	Sodium Benzoate
1	Linearity Range (µg/ml)	2.49-7.48	5.00-15.00
2	Correlation coefficient (R)	0.995	0.995
3	y-intercept	26449.6	39842.5
4	Slope	5173.3	13313.2

Precision Assessment

System precision was determined by performing the 6 replicates of standard PS and SB. The RSD of peak area was less than 2.0 % and performance of chromatographic system, represented by Retention time, number of theoretical plates and tailing factors were found well within the acceptable limits. The results are shown in Table 6.

In terms of method precision, the RSD of assay results for PS and SB in evaluation of repeatability, intermediate precision and overall RSD of 12 samples was less than 2.0 %, as shown in Table 7, 8. Therefore, the results showed that the method is precise.

Table 6: Results of System precision parameter.

Sr. No.	Name of Standard	Retention time	Theoretical Plates	Tailing Factor	% RSD
1	PS	10.93	5645	1.06	0.4
2	SB	13.95	5514	1.04	0.2

Table 7: Results of Method precision parameter.

Sr. No.	Name of Analyte	Mean Assay (%)	% RSD
1	PS	0.0997	0.6
2	SB	0.2011	0.5

Table 8: Results of Intermediate precision parameter.

Sr. No.	Name of Analyte	% RSD for Set-1	% RSD for Set-2	Overall % RSD
1	PS	0.6	1.8	1.4
2	SB	0.5	1.1	0.8

Accuracy Assessment

The accuracy study was carried out by spiking known amount of standards into placebo solution at 50 %, 100 % and 150 % of working concentration, respectively. The overall recovery percent were calculated. The results of recovery studies gave the recovery rate from 98.5 % to 101.6 % for PS and 98.6 % to 100.8 % for SB at all three levels for all the two analytes. The details are mentioned in Table 9 and 10.

Table 9: Results of Accuracy Study for Potassium sorbate.

Recovery level	Spiked Conc. (µg/ml)	Mean Area	Recovered Conc. (µg/ml)	% Recovery
(50 %) Level_1_1	2.49	76820	2.50	100.4
(50 %) Level_1_2	2.49	77730	2.53	101.6
(50 %) Level_1_3	2.49	76275	2.48	99.6
(100 %) Level_2_1	5.18	156995	5.11	98.6
(100 %) Level_2_2	5.08	157338	5.12	100.8
(100 %) Level_2_3	5.08	157513	5.13	101.0
(150 %) Level_3_1	7.68	238845	7.77	101.2
(150 %) Level_3_2	7.78	238072	7.75	99.6
(150 %) Level_3_3	7.88	238610	7.76	98.5

Table 10: Results of Accuracy Study for Sodium benzoate.

Recovery level	Spiked Conc. (µg/ml)	Mean Area	Recovered Conc. (µg/ml)	% Recovery
(50 %) Level_1_1	5.10	221378	5.03	100.8
(50 %) Level_1_2	5.00	221645	5.04	100.6
(50 %) Level_1_3	5.00	221341	5.03	100.4
(100 %) Level_2_1	10.10	446125	10.14	98.6
(100 %) Level_2_2	10.30	447307	10.16	99.0
(100 %) Level_2_3	10.20	444432	10.10	99.4
(150 %) Level_3_1	15.40	674087	15.31	99.7
(150 %) Level_3_2	15.40	675682	15.35	98.6
(150 %) Level_3_3	15.60	677571	15.39	98.7

Robustness Assessment

The robustness of RP-HPLC method was evaluated by analyzing the influence of minor modifications in HPLC conditions by varying the parameters like flow Rate (±0.2 ml/min), pH of mobile phase buffer (±0.2) and mobile phase composition (±2 %). The method is found to be robust as there is no such variation (RSD less than 2.0 %) was found in the results after alteration of the evaluated parameters. The details are given in Table 11.

Table 11: Results of Robustness.

Standard Solutions	Parameter condition	RT	Mean Area	SD	% RSD	Average % RSD
PS	**Flow Rate (± 0.2 ml/min)**					
	1.3 ml/min	12.72	147555	645.3075	0.4	0.5
	1.5 ml/min	10.93	150377	641.7779	0.4	
	1.7 ml/min	9.69	114628	939.3986	0.8	
	Mobile phase buffer pH (±0.2)					
	pH 2.3	10.06	140460	389.1767	0.3	0.5
	pH 2.5	10.93	150377	641.7779	0.4	
	pH 2.7	10.21	141345	1141.7518	0.8	
	Mobile phase composition (±2 %)					
	82:9:9	10.74	141254	648.5778	0.5	0.6
	80:10:10	10.93	150377	641.7779	0.4	
	78:11:11	9.40	143218	1148.6916	0.8	
SB	**Flow Rate (± 0.2 ml/min)**					
	1.3 ml/min	16.27	442523	533.5319	0.1	0.2
	1.5 ml/min	13.95	424645	713.7200	0.2	
	1.7 ml/min	12.40	340303	629.3933	0.2	
	Mobile phase buffer pH (±0.2)					
	pH 2.3	12.75	411196	1029.6530	0.3	0.2
	pH 2.5	13.95	424645	713.7200	0.2	
	pH 2.7	12.81	408538	829.5856	0.2	
	Mobile phase composition (±2 %)					
	82:9:9	13.69	413356	548.5892	0.1	0.2
	80:10:10	13.95	424645	713.7200	0.2	
	78:11:11	11.77	414897	825.6174	0.2	

CONCLUSION

The developed method is able to separate the potassium sorbate and sodium benzoate simultaneously with good resolution in herbal syrup formulation.

In this study, an RP-HPLC method has been developed and validated for simultaneous assay of preservatives PS and SB as per the ICH guidelines.

The method is found to be simple, suitable, specific, precise, accurate and robust, hence can be used for routine analysis in Quality Control Lab.

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