Investigation of Sodium Benzoate and Potassium Sorbate in Anti-Diabetic Herbal Drugs

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Abstract

The objective of the study was to assess sodium benzoate and potassium sorbate in some anti-diabetic herbal drugs (ADHDs) collected from local markets using UV-Vis spectrophotometric method. Analytes were measured in bulk and finished drug formulations at 224 nm for Sodium benzoate and 254.5 nm for potassium sorbate. The calibration curve obeyed Beer's law in the range of 0-40 ppm for sodium benzoate and 0-50 ppm for potassium sorbate and passed the goodness of fit evaluation. Limit of detection, LOD and limit of quantification, LOQ for sodium benzoate was 0.13 pm and 0.40 ppm respectively. The limit of detection, LOD and limit of quantification country is unknown. To conclude, whether the herbal drugs are safe to consume in terms of preservatives, more assessment is required., LOQ for potassium sorbate were 0.28 pm and 0.85 ppm respectively. No samples crossed safety limits for sodium benzoate and potassium sorbate. Overall picture of preservative content in herbal drugs of the country is unknown and, in this study, we identified both preservatives in the drug samples. To conclude, whether the herbal drugs are safe to consume in terms of preservatives, more assessment is required.

Keywords: *UV-Vis spectrophotometric method, sodium benzoate, potassium sorbate and anti- diabetic herbal drug.*

1. Introduction

Widespread escalation of type 2 diabetes has created worldwide anxiety, especially in developing countries, due to diabetic-related complications. As a result, along with allopathic drugs, herbal drugs are widely sought and consumed. However, herbal drugs do not go through similar procedures for quality control as their allopathic counterparts. In terms of safety assessment, investigation of preservatives in herbal drugs is hardly done in Bangladesh. Preservatives are added to delay the alteration and degradation of a drug from microbiological, enzymatic, or chemical changes, ensuring prolonged shelf life, preventing adverse changes, rotting of drugs, suppressing the proliferation of microorganisms and preserving drug quality [1]. Sodium benzoate and potassium sorbate have commonly been used as a preservative in pharmaceutical formulations [2- 4]. These

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preservatives are generally recognized as safe (GRAS) and can be used if mentioned at the product level. If the safety limit is exceeded then in short term, they can exert mild symptoms like irritation on the eyes, skin, and the respiratory tract upon short-term exposure. However, repeated, long-term exposure can result in urticaria, asthma, and contact dermatitis [2]. Therefore, there is increasing interest in the quantification of the preservative in finished formulations [5].

Diabetes is one of the top ten fatal diseases [6] and deaths due to diabetes-related complications are increasing. This horrendous scenario is traded by electronic and print media as a way to attract consumers to focus on herbal drug consumption for treating the ailment [7]. Consumption of herbal drugs for the treatment, prevention, and management of different diseases is common in Rajshahi City, Bangladesh where anti-diabetic herbal drugs are the leading sold drugs. This consumption requires a proper quality check of these remedies from manufacturing to selling. However, there remains doubt about the quality checks of these preparations. This dubiety could endanger public health safety. No work on preservative analysis has been done on anti-diabetic herbal preparations in this region. An investigation of such would be helpful to both the local people and the scientific community. Therefore, the present study aimed to determine the level of sodium benzoate and potassium sorbate in anti-diabetic herbal drugs.

2. Methodology

2.1. The Study area

In total, forty-eight anti-diabetic herbal drug samples were collected from herbal drugs and product selling hotspots consisting of drug selling outlets and superstores. Procured drugs were from five different manufacturers. Each manufacturer was selling several drugs belonging to the anti-diabetic class. Drugs that fall under each manufacturer were coded in numeric order: 1,2,3 and so forth. Herbal drugs were sold as finished commercial packs. After collection, samples were transported to a research laboratory and preserved as per the written direction on the packaging wall.

2.2. Apparatus

A UV- Vis Spectrophotometer with low stray light (0.5 % max) and ultra-fast scanning (29000 nm/ min) (Shimadzu 1900i, Shimadzu Corporation, Kyoto, Japan) was used for concentration measurements of standard and anti-diabetic herbal drugs. The apparatus was equipped with a 10 mm quartz cell matched pair. An electronic balance (Shimadzu ATY 224) with good precision (≤0.1mg) and linearity (±0.2mg) was used for weighing purposes.

2.3. Reagents

Analytical grade sodium benzoate and potassium sorbate (Scharlab S.L., Spain, 99-100% claimed purity into its certification) was purchased from a local supplier.

2.4. Methodology

2.4.1 Preparation of standards

Accurately weighed 100 mg of analytes (sodium benzoate/ potassium sorbate) was added in a 1000 mL volumetric flask and dissolved in water to obtain a 100 ppm standard stock solution. From this standard stock solution, working standards of different concentrations for sodium benzoate and potassium sorbate were made.

2.4.2 Construction of calibration curve

At first, the absorbance of solvent was fixed at zero using the auto-zero function of the spectrophotometer. A quick scanning (400-190 nm) of one of the working standards gives the wavelength with maximum absorption λmax known as the analytical wavelength for performing the rest of the concentration measurements at that wavelength. Standard solutions of different concentrations were run to the spectrophotometer, resulting in their respective absorbances. A calibration curve was plotted from the concentration vs absorbance diagram.

2.4.3 Preparation of formulations

Tablet formulations were macerated for 72 hrs with occasional shaking after weighing and adding 80 mL of deionized water [8- 11]. The heterogeneous mixture was filtered to remove particulate matter from the samples and the filtrate was collected in a 100 mL volumetric flask. To ensure extraction of the analyte, water was poured over filter paper and the washing was added to the volumetric flask to make up for the mark. The homogeneous mixture was diluted 100 times and an aliquot was taken in a sample cell to record instrument response.

Exactly one gram of the liquid formulation was weighted in a 1000 mL volumetric flask and 80 mL of deionized water was added, followed by maceration for 72 hrs with occasional shaking. The heterogeneous mixture was filtered to remove particulate matter from the samples and the filtrate was collected in a 100 mL volumetric flask. To ensure extraction of the analyte, water was poured over filter paper and the washing was added to the volumetric flask to make up for the mark. The homogeneous mixture was diluted 100 times and an aliquot was taken in a sample cell to record instrument response.

2.5. Percentage of analytes in anti-diabetic herbal drugs

Solid dosage formulation: The quantity of sodium benzoate in herbal drugs was determined utilizing the following formula:

Daily Exposure (DE) = (C_{HD} x W_{HD} x E)/ 1000 mg [12]

Where,
C_{HD} = Concentration of analyte in mg in drug
W_{HD} = Weight of herbal drug in mg
E=Number of exposures per day.

The validation of the developed method was carried out based on parameters including linearity, sensitivity, precision and accuracy as per ICH guidelines [13,14].

2.6. Linearity study

For linearity study, prepared working standards were added in the sample cell without changing the water in the reference cell. Concentration data with respective absorbance was obtained. The data sets were transformed into a calibration curve.

2.7. Precision study

Repeatability and intermediate precision of the developed method were studied by intra-day and inter-day precision. For intra-day precision estimation, different working standards were prepared

in triplicates at three different times of the same day and respective absorbances against concentrations were documented. The related standard deviation (RSD) was computed.

2.8. Accuracy study

The accuracy of the analytical method was studied by spike and recovery (SAR) assessment. Spiking was done in the analyte free plant matrix. Neem plant leaf was chosen. Three healthy leaves were cleaned, ground using mortar and pestle and suspended in water. The suspension was filtered and the filtrate was used for the accuracy study. Spiking of different concentrations was added to the plant matrix and concentration was measured. All measurements were done in triplicates and the result was shown as mean± SD value.

2.9. Method sensitivity

Method sensitivity was assessed according to ICH guidelines. Limit of detection (LOD) and limit of quantification (LOQ) can be defined by the following formulas:

LOD= (3 x σ)/ s
LOQ= (10 x σ)/ s

Here, σ is the standard deviation of the response of the blank and *S* is the slope of the analytical curve. LOD and LOQ were determined from the slopes of calibration curves and the standard error of regression equations.

3. Results and Discussion

Sodium benzoate and potassium sorbate are freely water-soluble. Therefore, water has been used as the diluent and used as a blank during measurement by UV-Vis spectroscopy. The analytical wavelengths of maximum absorptions (λmax) were 224 nm and 254.5 nm for sodium benzoate and potassium sorbate respectively (Fig. 1).

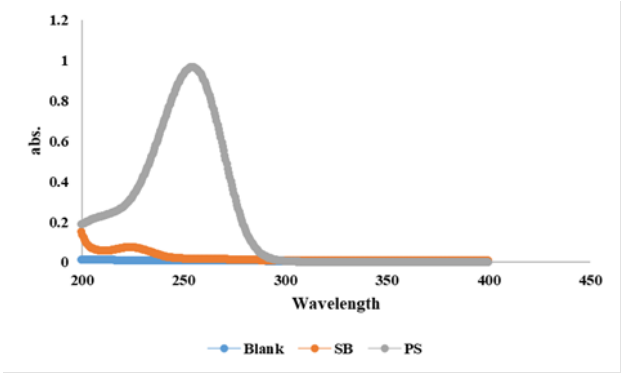

Fig. 1 Spectrum for blank, Sodium benzoate (SB) and potassium sorbates (PS).

Daily exposure to sodium benzoate and potassium sorbate in 48 anti-diabetic herbal drugs from different manufacturers can be seen in Table 1. For sodium benzoate, the International Programme on Chemical Safety sets a maximum tolerable limit of 45.3 g/70 kg healthy person/ day [15]. With this ceiling, all drugs were found safe to consume. This high ceiling may be due to metabolization and elimination of the analyte in urine as hippuric acid or glucuronic acid for higher levels of intake. On the contrary, the maximum acceptable daily intake of potassium sorbate for

humans is 25 mg/ kg of body weight per day which for an adult of 150 pounds, stands at 1,750 mg per day [16]. None of the drugs under investigation cross this safety ceiling considering their maximum daily consumption.

Table 1: Daily Exposure to sodium benzoate and potassium sorbate

Sample Coding	Batch No	Sodium Benzoate		Potassium Sorbate	
		DE (mg/day)	PDE (mg/day)	DE (mg/ day)	PDE (mg/ day)
1	1	43.6	4503	23.82	1750
2	3	46.54	4503	27.58	1750
3	11	54.24	4503	51.3	1750
4	54	30.76	4503	5.36	1750
5	81	21.46	4503	8.08	1750
6	1	46.7	4503	28.02	1750
7	4	54.43	4503	128.56	1750
8	8	53	4503	26.22	1750
9	1	125.88	4503	45.84	1750
10	1	202.98	4503	76.26	1750
11	6	307.04	4503	15.94	1750
12	5	297.24	4503	28.54	1750
13	5	334.54	4503	26.62	1750
14	8	286.46	4503	22.92	1750
15	6	327.54	4503	29.08	1750
16	1	309.58	4503	52.8	1750
17	9	96.1	4503	53.16	1750
18	5	111.7	4503	57.16	1750
19	14	75.7	4503	11.38	1750
20	5	45.92	4503	10.92	1750
21	9	72.86	4503	22.18	1750
22	5	160.98	4503	51.26	1750
23	9	111.18	4503	31.66	1750
24	15	1081.4	4503	246.24	1750
25	1	62.8	4503	15.58	1750
26	2020-01/1(1)	6.52	4503	N/A	1750
27	2019-03/1(1)	1.88	4503	N/A	1750
28	2020-02/1(1)	5.08	4503	1.06	1750
29	2020-01/1(1)	10.78	4503	7.26	1750
30	2020-10/1(4)	12.96	4503	3.92	1750
31	1	27.12	4503	21.02	1750
32	1	28.7	4503	19.2	1750
33	3	19.1	4503	12.86	1750
34	2	15.48	4503	10.12	1750
35	2020-02/1(2)	15.02	4503	9.97	1750
36	2020-11/1(2)	38.22	4503	23.22	1750
37	2020-02/1(1)	9.84	4503	N/A	1750

38	2019-06/1(2)	12.26	4503	N/A	1750
39	2019-06/1(1)	46.6	4503	N/A	1750
40	12	132.16	4503	26.76	1750
41	20914001	30	4503	42.14	1750
42	LTZD	45.54	4503	8.64	1750
43	1	45.28	4503	71.38	1750
44	2	69.2	4503	43.5	1750
45	46	59.96	4503	28.16	1750
46	B01M21E22	43.45	4503	92.96	1750
47	1	25.65	4503	30.42	1750
48	1	235.3	4503	21.4	1750

DE= Dialy Exposure
PDE= Permitted Daily Exposure

To study the validity of the experiment as per ICH guidelines, a goodness-of- fit study was conducted to see how well the model fits a set of observations. Here, absorbances at 224 nm and 254.5 nm were plotted against calibration standards for sodium benzoate and potassium sorbate showing a relationship obeying Beer's law (Fig. 2 and Fig. 3).

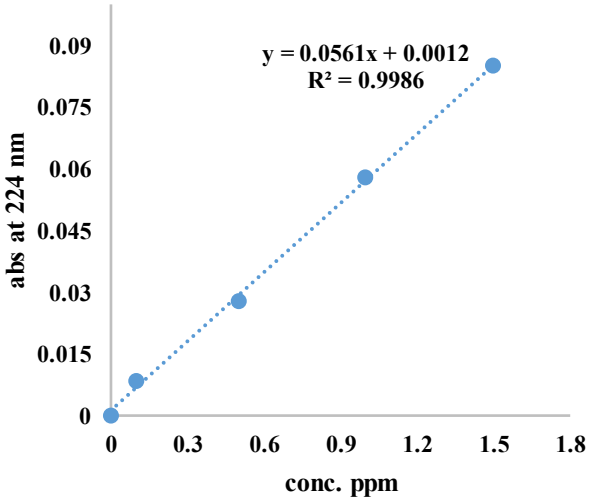

Fig. 2 Sodium Benzoate Calibration plot

The analytical curves were fitted by the least square method. Goodness to fit study was performed by the two tests; regression validation and test for linearity. Regression statistics were performed on absorbance and concentration data sets. The following regression model was found:

$A_{SB} = 0.0561 \times C_{SB} + 0.0012$ and
$A_{PS} = 0.0296 \times C_{PS} - 0.0014$.

Where,
A_{SB} = Absorbance of sodium benzoate
A_{PS} = Absorbance of potassium sorbate

C_{SB} = Concentration of sodium benzoate
C_{PS} = Concentration of potassium sorbate

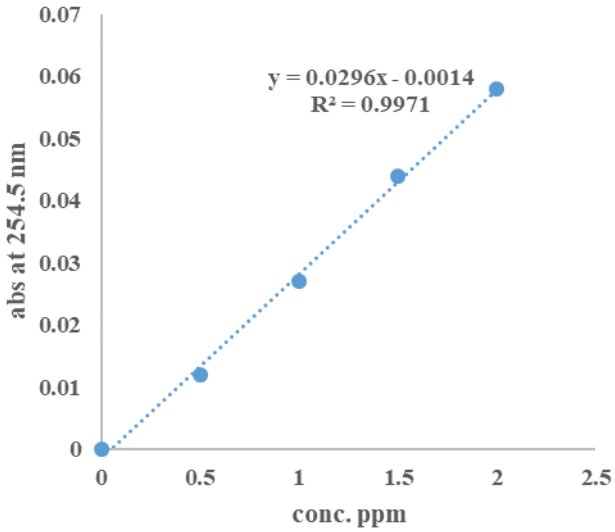

Fig. 3. Potassium sorbate calibration plot

The coefficient of variation R^2 is one measure of goodness-of-fit. Here, R^2 was found close to 1 for both analytes (0.9986 and 0.9971 for sodium benzoate and potassium sorbate respectively) (Fig. 2 and 3). However, this value does not guarantee that the model fits the data well as there is a problem with the R^2 as a measure of model validity. It can always be increased by adding more variables to the model. To overcome this difficulty, the F test was performed. Between-group variation was found significant at a 5% level of significance ($p<0.05$) for both analytes.

To find the relationship between the predictor (concentration) and response (absorbance) variables Pearson Correlation Coefficient (PCC), r was determined (Table 2). As Cauchy–Schwarz inequality puts the obtained PCC, r-value of 1 at perfect positive linear correlation, the purpose of additively is fulfilled. At a value of zero of the predictor variables (concentration), a value of 0 is obtained as a response value (absorbance), and homogeneity of the relationship is confirmed. When the requirement of additivity and homogeneity is fulfilled, the model is said linear which satisfies proportionality. Therefore, this model is validated as goodness–to–fit for the determination of sodium benzoate and potassium sorbate as an analyte in a water medium.

Table 2: Optical Characteristics of the method

Parameter	Analyte	
	Sodium benzoate	**Potassium sorbate**
Analytical wavelength for sodium benzoate (λ_{max}) nm	224	254.5
Regression Equation, y=mx+c	y=0.0561x+0.0012	y=0.0296x-0.0014
Pearson Product Moment Correlation coefficent, (pcc, r)	1	1
Coefficient of Determination, R^2	0.9986	0.9971
Limit of Detection (LOD) ppm	0.13	0.28
Limit of Quantification (LOQ) ppm	0.4	0.85

Precision was studied in terms of intra-day precision. On the same day, data were taken at two different times (T1 and T2), a few hours apart. Measurements of concentrations (10 ppm, 20 ppm and 40 ppm) were done as triplicates. Results are shown in Table 3 with relative standard deviation (RSD).

Table 3: Intra-day precision for sodium benzoate and potassium sorbate

Time	Concentration (ppm)	Sodium benzoate concentration (ppm)				Potassium sorbate concentration (ppm)			
		Reading 1	Reading 2	Reading 3	RSD (%)	Reading 1	Reading 2	Reading 3	RSD (%)
T1	10	9.83	9.98	9.98	0.87	9.79	9.85	9.95	0.82
	20	19.71	19.57	19.8	0.59	19.85	19.85	19.78	0.20
	40	39.88	40.12	39.75	0.47	40.04	40.1	39.85	0.33
T2	10	9.77	9.85	9.95	0.91	9.83	9.88	9.98	0.77
	20	19.8	19.85	19.8	0.15	19.65	19.57	19.8	0.59
	40	40.04	40.1	39.95	0.19	39.88	40.1	39.75	0.44

To verify the accuracy of the method, a recovery study was conducted. Satisfactory recovery was observed for analyte spiking in preservative-free neem plant matrix where 88.4-105.8% sodium benzoate was recovered and 95-104.9% potassium sorbate was recovered (Table 10). All of this information indicating the detection of sodium benzoate and potassium sorbate was unaffected by the interference of excipients present in the sample matrix (Fig. 4) [17]. Therefore, it can be concluded that there was no loss in response or increase of response due to ion suppression or ion enhancement respectively.

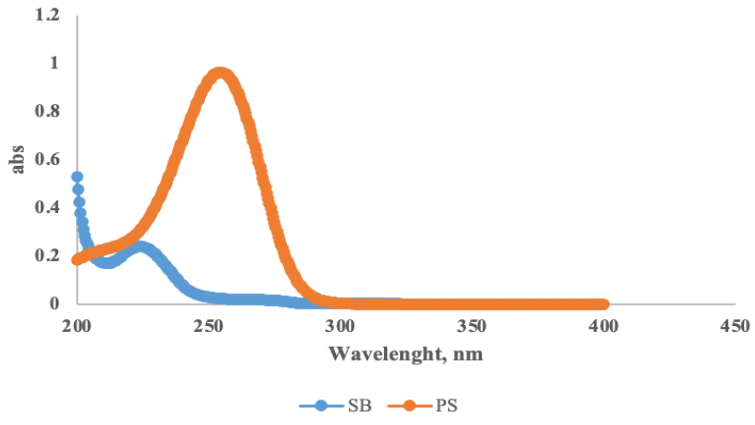

Fig. 4 Sample spectra for sodium benzoate (SB) and potassium sorbate (PS)

Sensitivity was studied in terms of limit of detection (LOD) and limit of quantification (LOQ). LOD and LOQ of the developed method were 0.13 ppm and 0.40 ppm respectively for sodium benzoate and LOD and LOQ of the developed method were 0.28 ppm and 0.85 ppm respectively for potassium sorbate (Table 2).

Table 4: Recovery of analytes in analyte free mix plant extract

Analyte spiked (ppm)	Recovery ppm(mean±SD)	
	Sodium benzoate	Potassium sorbate
5	88.40±0.00	104.80±0.00
10	96.80±0.00	104.60±0.00
15	97.70±0.00	104.90±0.03
20	100.20±0.00	102.30±0.23
25	105.80±0.00	95.00±0.29

4. Conclusions

As part of the safety assessment, 48 anti-diabetic herbal drugs from different manufacturers in Rajshahi City were assessed for sodium benzoate and potassium sorbate. For this purpose, UV-Vis spectrophotometric method was adopted. All of the samples were found to contain the analytes but within a safe limit. However, more assessment of the preservatives needs to be conducted on the herbal drugs to obtain the scenario of preservative content in herbal drugs of the country and its subsequent safety.

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