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PRESENCE OF METHYL PARABEN IN ANTI-DIABETIC HERBAL PREPARATIONS

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Abstract

Plant-based hypoglycemic therapeutics have been increasing in consumption due to the escalation of type 2 diabetes and its related complications. However, the safety of the preparations is little understood. Parabens (alkyl esters of p-hydroxybenzoic acid) are widely used as preservatives in these pharmaceuticals. However, the presence of methylparaben in formulations raises anxiety due to its potential endocrine disruption functions. Endocrine disruption could lead to undesirable health abnormalities and carcinogenic, estrogenic, and adverse reproductive effects. The present investigation directs toward estimating of methylparaben in some anti-diabetic herbal preparations using UV- Vis spectrophotometric method abiding by International Conference on Harmonization (ICH) guidelines for validation. The analytical wavelength of methylparaben in methanol was determined and found at 256.5 nm. The method obeys Beer's law in the analytical range and has a good coefficient of determination (r^2= 0.9881). The limit of detection (LOD) and limit of quantification (LOQ) were 0.19 ppm and 0.57 ppm, respectively. Recoveries were 91.3-98.8% in analyte-free plant matrix and 91-105.8 % in a diluent. The coefficient of variation (CV%) varied between 0.005-0.268% for different standards. Results of forty-eight anti-diabetic herbal preparations showed methylparaben was detected in thirty-four samples in the range of 13.12 – 325.13 mg/day with a mean exposure value of 78.25 mg/day. However, none of the samples raise concerns about safety (the safety ceiling for paraben is 420 mg/day). More investigation is required to determine, whether the herbal drugs are safe to consume in terms of methylparaben.

Key words: Methylparaben, UV-Vis spectrophotometric method, Safety assessment.

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Introduction

Prolonged shelf life is an indication of drug quality. Shelflife is affected by the modification and degradation of a drug due to microbiological, enzymatic, or chemical changes. Preservatives delay or restrict the process (Malik *et al.*, 2010; Mahboubi *et al.*, 2014). However, they can exert an adverse health effect in excess (Andersen *et al.*, 2019; Soni *et al.*, 2002; Nair 2001; Hannuksela *et al.*, 1987; Juhlin 1981; Lahti *et al.*, 1987; Rademaker *et al.*, 1989, Safford *et al.* 1990). The use of methylparaben in pharmaceutical preparations as a preservative is common (Mahboubi *et al.*, 2014). The preservative hails from the alkyl esters of p-hydroxybenzoic acid (parabens), which are a group of a homologous series of chemicals (Fig. 1). Properties of methylparaben like broad activity against bacteria, yeasts, and molds, no apparent odor and taste and chemical stability are thought playing a role behind their application in pharmaceuticals as preservatives (Soni *et al.*, 2002, Anderson *et al.*, 2005; El Hussein *et al.*, 2007). After entering the body parabens quick absorption occurs in the gastrointestinal tract and blood (Darbre *et al.*, 2004; Darbre *et al.*, 2008; Soni *et al.*, 2001).

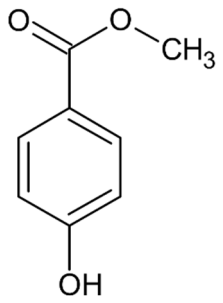

Fig. 1. Chemical structure of methylparaben.

The osteogenic effect of paraben is not mysterious (Routledge *et al.*, 1998). The effect has an association with female breast cancer incidence and the development of male reproductive system malignant melanoma (Tavares *et al*., 2009). Moreover, there are estrogenic effects on children if they consume parabens through drugs (Prudal *et al.*, 2015). The concern is also raised about the safe use of parabens due to their potential endocrine disruption properties in different *in-vitro* and *in-vivo* investigations (Miller *et al.*, 2001; Okubo *et al,*. 2001; Byford *et al.* 2002; Darbre *et al.*, 2008; Boberg *et al.* 2010; Witorsch and Thomsa, 2010). Endocrine disruption coupled with reproductive toxicity related to paraben raises the question about their widespread exposure, thereby

attracting substantial attention by national and international regulatory authorities (Toxicological evaluation of certain food additives with a review of general principles and of specifications, 1974; Scientific Committee on Consumer Safety, 2011). Regulatory authorities ask for data on the quantity of the preservatives in different drugs and foods (CFR, 1999). Therefore, quantification of the analyte in finished drugs must be required.

Rajshahi City is a northern metropolis of Bangladesh. Along with the increasing number of diabetic patients, the consumption of anti-diabetic herbal preparations is increasing. No herbal drug manufacturer provides information on its preservative status. Consequently, the amount of consumption of parabens through herbal formulations has yet to be discovered. If excess parabens are consumed through the preparation, then drug safety could be breached, and an assessment of parabens is becoming mandatory. Therefore, in the current investigation, an assessment of the safety of some anti-diabetic herbal preparations in terms of methylparaben was conducted. Along with chromatographic methods (Sohrabvandi *et al.,* 2015; Baranowska *et al.,* 2004; Can *et al.,* 2011; Yan *et al.,* 2012; Ma *et al*., 2012), UV-Vis spectroscopic methods are common for analyzing preservatives (Mahboubifa *et al.,* 2010). An easy, accurate, and sensitive UV-Vis spectrophotometric analytical method was used to determine paraben concentrations in the herbal preparations. This study helps the local people and the scientific community. The herbal manufacturers can also use the analytical procedure in their lab for routine analysis of parabens.

Materials and Methods

Sample: In total, forty-eight anti-diabetic herbal preparations were procured from different manufacturers, and finished formulations bearing drug administration registration no (DAR No.) were chosen. The location of the manufacturing outlets shows similar characteristics; the collection points were densely populated areas where consumers purchased drugs from their close neighborhood, and the collection points also acted as a junction through which mass movement of people takes place. When returning home from the city, the consumers purchase drugs from the outlets. After collecting samples from shops, they were taken to the research laboratory, Department of Chemistry, University of Rajshahi, where information attached to the drugs (either in the package insert or on the packaging wall) recorded in tabulated form. The drugs were then blindfolded by coding to avoid bias before the assay (Table 1).

Table 1. Sample information.

Sample Code	Batch No.	DAR No.	Manufacturer	Max. dosage/ day	Unit drug wt. (g)
1	1	U-038-A-033	1	2 cap 2 times	0.58
2	3	U-038-A-020	1	2 tab 2 times	0.56
3	11	U-038-A-094	1	3 cap 2 times	0.58
4	54	U-038-A-029	1	2 tab 2 times	0.41
5	81	U-038-A-028	1	2 tab 2 times	0.40
6	1	U-038-A-017	1	2 tab 3 times	0.57
7	4	U-038-A-021	1	2 tab 2 times	0.57
8	8	U-038-A-018	1	2 tab 1 times	0.57
9	1	U-038-A-100	1	4 cap 3 times	0.54
10	1	U-038-A-074	1	1 tab 2 times	0.96
11	6	H-82-A-61	2	2 tab 2 times	0.64
12	5	N/A	2	10 gm 3 times	0.62
13	5	H-82A-054	2	5 tab 3 times	0.59
14	8	H-47A-061	2	2 tab 2 times	0.66
15	6	003-02-94	2	2 tab 3 times	0.65
16	1	003-0002-94	2	2 cap 2 times	0.62
17	9	H-82A-039	2	2 cap 3 times	0.50
18	5	Ayu-210A-007	2	3 cap 2 times	0.46
19	14	H-82A-028	2	3 tea spoon 3 times	1.08
20	5	U19-A-128	3	2 tab 3 times	0.64
21	9	U-19-A-040	3	2 tab 2 times	0.63
22	5	U-19-A-219	3	1 cap 2 times	0.64
23	9	015-0005-94	3	1 cap 2 times	0.60
24	15	Ayu-78A-019	3	1 sachete 3 times	3.83
25	1	015-14-94	3	2 cap 2 times	0.61
26	2020-01/1(1)	Ayu-4A-013	4	3 tab two times	0.28
27	2019-03/1(1)	Ayu-4A-011	4	3 tab two times	0.28
28	2020-02/1(1)	Ayu-4A-384	4	3 tab two times	0.23
29	2020-01/1(1)	Ayu-A-014	4	3 tab two times	0.44
30	2020-10/1(4)	Ayu-A-121	4	3 tab two times	0.64
31	1	Ayu-4A-058	4	4 tea spoon 2 times	0.02
32	1	Ayu-4A-061	4	4 tea spoon 2 times	0.02
33	3	Ayu-4A-063	4	4 tea spoon 2 times	0.02
34	2	Ayu-4A-119	4	4 tea spoon 2 times	0.02
35	2020-02/1(2)	N/A	4	3 tab two times	0.50
36	2020-11/1(2)	Ayu-A-301	4	3 tab two times	0.46
37	2020-02/1(1)	Ayu-A-318	4	3 tab 2 times	0.22
38	2019-06/1(2)	Ayu-A-310	4	3 tab 2 times	0.25
39	2019-06/1(1)	Ayu-A-309	4	3 tab 2 times	0.50
40	12	Ayu-18A-054	5	1 cap 3 times	0.57
41	20914001	U-137A-027	5	2 cap 2 times	0.60
42	LTZD	U-114A-006	5	2 tab 2 times	0.68
43	1	U-124-A-38	5	2 tab 2 times	0.53
44	2	U-17A-027	5	2 cap 2 times	0.52
45	46	U-301A-051	5	2 tab 2 times	0.60
46	B01M21E22	Ayu-26A-019	5	1 tab 2 times	0.57
47	1	5A-144	5	1 tab 2 times	0.57
48	1	U308A13	5	2 cap 3 times	0.60

Tab= tablet, cap= capsule, DAR No.= Drug administration registration number

Apparatus used

- A UV- Vis Spectrophotometer with low stray light (0.5 % max) and ultra-fast scanning (29000 nm/min) (Shimadzu 1900i, Shimadzu Corporation, Kyoto, Japan)
- An electronic balance (Shimadzu ATY 224) with good precision (≤ 0.1mg) and linearity (±0.2mg)

Chemicals used

- Analytical grade methylparaben (Scharlab S.L., Spain, 99-100% claimed purity into its certification)
- Methanol (assay above 99.99%) manufactured by Merck, Germany.

Standard stock solution and calibration standard preparation

Accurately weighed 100 mg of methylparaben and poured it into a 100 ml volumetric flask. Methanol was added, and the volume was made up to the mark. A 1000 ppm standard stock solution is ready. From this standard stock solution, calibration standards (0 ppm, 1 ppm, 2 ppm, 3 ppm, and 4 ppm) were prepared. Analytical wavelength (λ_{max}) was taken and found at 256.5 nm (Fig. 2).

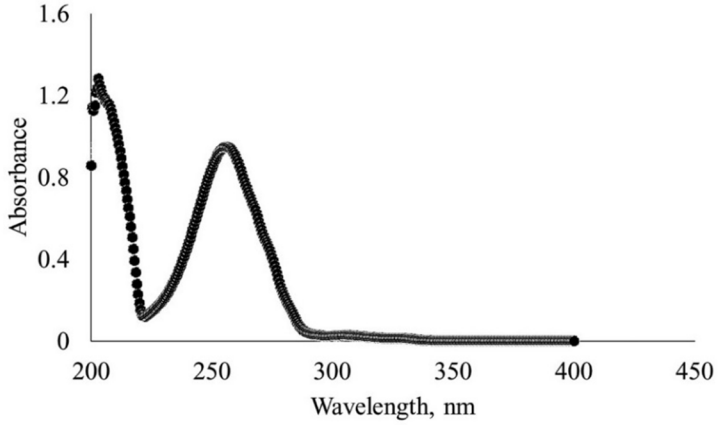

Fig. 2. Analytical wavelength (λ_{max}) determination for methylparaben.

Analysis of samples: Herbal drug samples (one piece for each solid sample and one gram for liquid sample) were macerated for two days with occasional shaking in 50 mL of methanol (Ingle *et al.* 2017, Azwanida 2015, Pandey and Tripathi *et al.* 2014, Doughari

2012). Capsule drugs were holed to escape the inner material into the solvent. The heterogeneous mixture was filtered, and the filtrate was collected. The filtrate was diluted 100 times (0.1 ml filtrate + 9.9 ml methanol). Solvent (methanol) was taken in both cells (sample cell and reference cell) of the spectrophotometer and made auto-zero. A calibration curve was obtained by replacing the solvent in the sample cell with calibration standards one after another in lower to higher concentrations. Then analyte aliquot was added to the sample cell. An instrument response was recorded. 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 (Islam *et al.* 2022, Alhusban and Sawsan *et al.* 2019)

Where,

C_{HD} = Concentration of analyte in mg in drug

W_{HD} = Weight of herbal drug in mg

E=Number of exposures per day.

Method validation: The validation of the utilized method was carried out based on linearity, sensitivity, precision, and accuracy as per ICH guidelines (ICH, 1995A, ICH 1996 B). Calibration and validation sets were shown (Table 2).

Table 2. Calibration and validation sets.

Calibration sets (mg/L)	Validation sets (mg/l)		
	Accuracy Study		Precision study
	Plant matrix	Diluent	
0	3	10	10
1	4	15	15
2	5	20	20
3	6	25	25
4	7	30	30

Linearity study: For the linearity study, calibration standards were added to the sample cell without changing the solvent (methanol) in the reference cell. Concentration data with respective absorbance was obtained and the data sets were transformed into a calibration curve (Fig. 3).

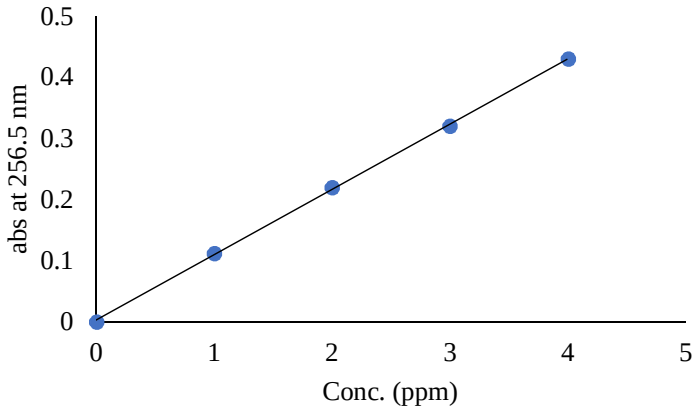

Fig. 3. Calibration plot for methylparaben.

Accuracy and precision study: The accuracy of the analytical method was studied by spiking known analytes in a diluent and analyte-free plant matrix. The sample was split into two portions, namely spiked and unspiked portions. Five different standard solutions containing analytes were added to the diluted sample solution in the spiked portion. The concentration of analytes was measured in both spiked and unspiked portions.

Recovery was calculated as,

$$\%R = \frac{C_S - C_U}{C_A} \times 100$$

Where,

R = Recovery

C_S= Analyte concentration in the spiked portion

C_U= Analyte concentration in the unspiked portion

C_A= Concentration of analyte added.

Additionally, spiking was done in diluent and preservative-free plant extract.

Method sensitivity: Method sensitivity was assessed according to ICH guidelines. The limit of detection (LOD) and limit of quantification (LOQ) were determined by the following formulas,

$$LOD = {(3.3 \times \sigma)}/{S}$$

$$LOQ = {(10 \times \sigma)}/{S}$$

Here, σ is the standard deviation of the response of the blank and S is the slope of the analytical curve.

Result and Discussion

Methylparaben is methanol soluble, used as a blank and solvent during measurement by UV Vis spectroscopy. The analytical wavelength (maximum wavelength, λ_{max}) was 256.5 nm (Table 3).

Table 3. Optical Characteristics of the method.

Parameter	Value
Analytical wavelength for methyl paraben (λ_{max}) nm	256.5
Concentration range obeys Beer's law (ppm)	10-30
Regression Equation, y=mx+c	$y = 0.106 \times x + 0.0028$
Slope	0.106
Intercept	0.0028
Coefficient of Determination, R^2	0.9881
Limit of Detection (LOD) ppm	0.19
Limit of Quantification (LOQ) ppm	0.57

Linearity test: To find the relationship between the predictor (concentration) and response (absorbance) variables, Pearson's Correlation Coefficient (PCC), r was determined. As the 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 the response value (absorbance) and the homogeneity of the relationship is confirmed. When the requirements of additivity and homogeneity were fulfilled, the model was said to be linear, which satisfies proportionality.

Accuracy and precision study: A recovery study was performed to verify the accuracy of the applied method. The known concentration of analyte was added to a methylparaben - free plant-matrix (diluted) and then directly into the diluent. Recovery was found within the satisfactory level (91.3-98.1%) in mixed plant matrix and (91-105.8%) in direct diluent (Andreasson, 2015) (Table 4). All of this information indicates that the detection of the analyte was unaffected by the interference of excipients present in the plant matrix (Lee *et al.* 2006).

Table 4. Recovery study.

Analyte free plant matrix		Diluent	
Spiked amount ppm	Recovery %	Spiked amount ppm	Recovery %
3	91.3	10	105.8
4	98.1	15	91.0
5	96.9	20	103.3
6	95.2	25	101.8
7	95.2	30	96.5

To assess precision, different standards (10 ppm, 15 ppm, 20 ppm, 25 ppm, and 30 ppm) were prepared and the coefficient of variation was found in the range of 0.005-0.268% (Table 5), indicating good precision.

Table 5. The precision of analytical data.

Standard ppm	Mean conc. found (ppm) n=3	Co-efficient of variation (CV)%
10	10.58	0.005
15	13.64	0.046
20	20.66	0.074
25	25.45	0.191
30	28.96	0.268

Sensitivity: Sensitivity was studied as the limit of detection (LOD) and limit of quantification (LOQ). The LOD and LOQ of the developed method were 0.19 ppm and 0.57 ppm, respectively, for methylparaben (Table 3).

Safety assessment of anti-diabetic herbal drugs: Methylparaben is an alkyl ester of p-hydroxybenzoic acid. The analyte is readily absorbed from the gastrointestinal tract after oral administration. The ester is hydrolyzed to its reactant molecule, para hydroxy benzoic acid. Then it is excreted through urine without accumulating in the body. However, studies showed, allergic reactions upon oral paraben exposure (Soni et al., 2002). Daily exposure to the ester is shown (Fig. 4). The safety limit per day for a 70 Kg human being is 420 mg. With this value, no instances crossed safety (Final Report on the Safety Assessment of Methylparaben, Ethylparaben, Propylparaben, and Butylparaben, 1984).

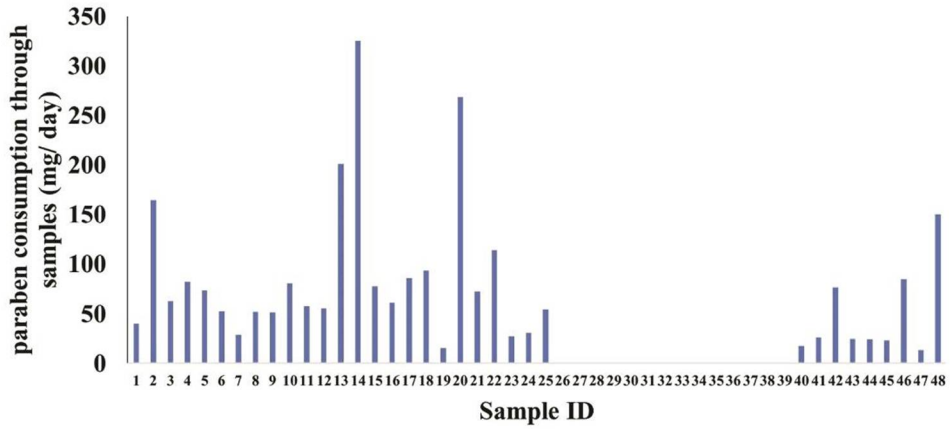

Fig. 4. Daily methylparaben consumption through anti-diabetic herbal preparations

Conclusions

As part of the safety assessment, forty-eight anti-diabetic herbal drugs from different manufacturers in Rajshahi City were screened for methylparaben using a UV-Vis spectrophotometer. Thirty-four out of forty-eight samples were found to contain the analytes but within a safe limit. As the method was found to be faster, more precise, and cheaper, the analytical procedure can be used for routine analysis of parabens in herbal drugs and other preservatives in the manufacturers' labs. More assessment of the preservatives needs to be carried out on the herbal drugs of other parts of Bangladesh to obtain the scenario of preservative content in the country and its subsequent safety.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgment

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