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Research article

Microbial and physicochemical evaluations of paracetamol in different brands of analgesic syrups sold in Bangladesh

Netish Kumar Kundo[1*], Chand Sultana[1], Mohammad Sayful Islam[1], Md. Ashraf Ali[1], Marina Khatun[2], A. H. M. Mazbah Uddin[1], Pijush Kanti Roy[3], Rana Ahmed[1], Md. Mahabubur Rahaman[1], Md. Arafat Rahman[1]

[1]*Department of Pharmacy; Mawlana Bhashani Science and Technology University, Santosh, Tangail-1902, Bangladesh.*
[2]*Department of Biochemistry and Molecular Biology; Mawlana Bhashani Science and Technology University, Santosh, Tangail-1902, Bangladesh.*
[3]*Department of Chemistry; Mawlana Bhashani Science and Technology University, Santosh, Tangail-1902, Bangladesh.*

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***Corresponding Author: Netish Kumar Kundo,** Department of Pharmacy, Mawlana Bhashani Science and Technology University, Santosh, Tangail-1902.

Abstract

Different brand of paracetamol syrups are widely used in Bangladesh, especially for pediatric and aged patients. The therapeutic efficacy of pharmaceutical products depends on both microbial and physicochemical qualities of the products. However, liquid dosage forms especially syrups contain high percentage of sucrose that are more susceptible for microbial growth. This investigation was done to evaluate the microbial and physicochemical qualities of two hundred fifty samples of different brands of paracetamol syrups sold in Bangladesh. These brands were investigated according to the in-vitro compendial requirements, which include organoleptic properties such as color and taste, total viable aerobic count, type of isolated microorganisms, physical properties and content of active ingredients. These tests were performed by standard methods and techniques. The investigated five brands of paracetamol syrups were passed the standards of the USP regarding microbial specifications. The physiochemical qualities such as organoleptic test results had red and pink clear liquid with sweet taste in some analgesic syrups but remaining samples are bitter taste that not suitable for pediatric patients. The pH values were ranged from 4.44-5.88. However, the density fluctuated from 1.149-1.184 g/mL. The paracetamol concentration as an active ingredient in the paracetamol syrup was recorded from 94.1% – 103.5%. These findings explained that the five different brands of paracetamol syrups sold in Bangladesh are comply with Pharmacopeia specifications regarding microbial and physicochemical characteristics but for better patient acceptability especially for pediatric patients the taste of some paracetamol syrups should be improved.

Introduction

In the earlier of the 21st century, microbial contamination of non-sterile drugs is one of the main problems for product recalls and production slowdowns [1].

The presence of microbial contaminants was not only found to cause physicochemical changes that led to the spoilage of numerous products but was also proved to be a potential health hazard to the consumer. Non-sterile dosage forms are not required to be sterile, as recommended by most pharmacopeias, but are required to pass microbial bioburden tests for the absence of certain specified indicator pathogens (*Escherichia coli, Salmonella* sp., *Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida albicans*) to ensure their efficacy and safety [2].

Not only the presence of pathogenic microorganisms but the presence of relatively high number non-pathogenic microorganisms is also objectionable in pharmaceutical products. The presence of the large number of non-pathogenic microorganisms in pharmaceuticals is objectionable for two reasons: firstly, these microorganisms can deteriorate active ingredients and can interfere with the desired activity of the product; and secondly, they can produce some metabolites that may be toxic to the consumer [3, 4].

Some oral pharmaceutical drugs, if stored in a favorable environment, can serve as nutrients source for microorganisms. Humidity and high amount of sugar in the oral- liquid drugs-in particular can support the microbial growth. Oral liquid drug formulations such as aqueous solutions, suspensions, emulsions and syrups used in pediatrics are at a more risk of microbial contamination during use due to sweetening agents, reconstitution methods, unsuitable storage and handling defects. Microbial contaminations may ultimately contribute to secondary bacterial infections in pediatric patients [5, 6].

Microbial infections are not only the result of the physical presence of microorganisms, but also their metabolites/ toxins that become harmful even if they are found in minimal quantities. Some of these toxin-related illnesses include acute gastroenteritis, abdominal discomfort, and diarrhea [5, 7].

The nature of the active ingredients, the quality of the vehicle and the attention and attitude of workers involved in their handling influence the incidence of microflora in non-sterile products [18]. The nutrients availability, presence of microorganisms, and oxygen consider the some factors for extent of microbial contamination in non-sterile products [8].

The microbiological quality of pharmaceutical products mainly depends on the quality of raw materials, manufacture process and environment, hygiene of the personnel involved in the manufacture and the storage conditions [9].

The present study was designed to determine the percentage content of paracetamol, physical properties and the microbial contaminants found in some brands of analgesic syrups marketed in Bangladesh.

Materials and methods

Samples collection

Two hundred and fifty samples of analgesic syrups of five different companies having different manufacturing date were collected from various retail pharmacies in Tangail City, Bangladesh and labeled with the code A, B, C, D, and E.

Physical examinations

The color was assessed in each sample by visual examination, whereas the taste was evaluated by using the appropriate, relevant sense organs. The pH value was measured once by a pH meter instrument (Model: HI991001 Waterproof Portable pH Meter). The density measured by density measuring instrument.

Microbiological examination

Total viable aerobic count

Ten mL of the sample was diluted to bottle contained 90 mL of nutrient broth and mixed well. A quantity of 0.1 mL of the diluted sample was spread on the surface of Casein soya bean digest agar and Sabouraud dextrose agar plates. The casein soya bean digest agar plates were incubated at 35°C for three days while the Sabouraud dextrose agar plates were incubated at 25°C for five days with daily observation. All experiments were done in duplicates and controls set up in each round. Colonies were counted, and the mean number of colony forming units per mL of each syrup was calculated and recorded [19].

Identification of isolated microorganisms

The sample of the syrups was placed on various selective media such as MacConkey agar, Sabouraud dextrose agar, Baird Parker agar and xylose lysine deoxycholate and then incubated. The biochemical tests used were oxidase, catalase, methyl red (MR), Voges-Proskauer (VP), motility indole ornithine decarboxylase (MIO), triple sugar iron (TSI) citrate utilization, urea and mannitol fermentation. Gram staining and Lactophenol cotton blue stain technique [20, 25-26].

Chemical examinations

Determination of paracetamol (PCM) content

Preparation of working standard solution

The standard procedure for preparation of PCM working standard solution for syrup containing 120 mg/5 mL. 120 mg of PCM working standard (Supplier: NIP chemicals, Batch No.: PFM-188-06-13, Retest date: 22-06-16) weight was diluted with 50 mL of 0.1 M sodium hydroxide (NaOH) into a volumetric flask (200 mL) and completed by distilled water (DW) to the volume. One mL of the diluted solution was transferred into a volumetric flask (100 mL) contained 10 mL of 0.1 M (NaOH) and completed with DW to a volume [11].

Preparation of sample solution

Five mL from each paracetamol syrup sample was transferred into a volumetric flask (200 mL) contained 50 mL of 0.1 M (NaOH) and completed with DW to a volume. One mL of the diluted solution was moved into a volumetric flask (100 mL) contained 10 mL of 0.1 M (NaOH) and completed with DW to a volume [11].

Sample assayed by UV/visible spectrophotometer

The absorbance of the prepared PCM solutions (working standard and sample) were measured in a UV/visible spectrophotometer (Model: UV-1800 Shimadzu Spectrophotometer) with bandwidth (1 nm), connected to a computer loaded with software, at a wavelength of 257 nm using a mixture of 10 mL of 0.1 M (NaOH) and 90 mL of DW as a blank. Quartz cell was used to measure absorbance of all the PCM solutions [11].

The analysis of results was made as follow:

Percentage (%) = (Absorbance of sample/absorbance of standard) × (concentration of standard/concentration of test) × 100.

Statistical analysis

The obtained data was subjected to statistical analysis of variance (ANOVA) using IBM SPSS statistics software (version 20.0, 2011). Differences in microbial count, isolated microbial, physical and chemical characters were compared ANOVA test. Values of $p<0.01$ was considered statistically significant.

Results

Physical Parameters Results

The results of physical parameters include the color, description, taste, pH, and density (g/mL) that obtained

from analyzed paracetamol syrup samples are listed in the Table 1.

Microbiological Examination Results

A table 2 shows the total viable aerobic count of microorganisms present in the analyzed paracetamol syrup samples.

Chemical Results

Figure-1 & Table-4 shows the PCM in the analyzed analgesic syrups samples. The ANOVA test showed that there was a significant difference in the paracetamol content results when compared between analgesic syrup samples with an individual confidence level of 99%.

Table 1. Physical parameters of the different brands of the paracetamol syrups.

Parameters	Paracetamol Syrup Brand Code					P value
	A	B	C	D	E	-
Color	Red	Red	Pink	Red	Pink	-
Description	Clear solution	Clear solution	Clear solution	Clear solution	Clear solution	-
Taste	Bitter sweet	Sweet	Bitter sweet	Sweet	Sweet	-
pH	5.26 – 5.32	4.82 – 4.90	5.58 - 5.64	6.12 – 6.18	4.98 – 5.06	<0.01**
Density (g/mL)	1.112 -1.116	1.110 – 1.113	1.083- 1.088	1.146 – 1.148	1.107-1.109	<0.01**

**The P value is statistically significant at the 0.01 level.

Table 2.Total viable aerobic count in the tested paracetamol syrup samples.

Brand code	Total bacteria (CFU/mL)	Total fungi (CFU/mL)	Acceptable limit (CFU/mL) (USP, 2007) Bacteria	Fungi	*P* value
A	<10 (94%) <100 (6%)	≤10 (100%)			
B	<10 (92%) <100 (8%)	≤10 (100%)			
C	<10 (90%) <100 (10%)	≤10 (100%)	$<10^2$	10^1	0.01*
D	<10 (82%) <100 (18%)	≤10 (100%)			
E	<10 (91%) <100 (9%)	≤10 (100%)			

* The P value is not statistically significant at the 0.01 level. Sample size: 50

Table 3. Number (No.) and percentage (%) of bacteria and fungi isolated from tested paracetamol syrup samples.

Microorganisms	Brands code										Total		P value
	A		B		C		D		E				
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
Bacteria													
Bacillus subtilis	3	6	4	8	2	4	1	2	5	10	15	7.5	>0.01*
Staphylococcus epidermis	1	2	0	0	1	2	0	0	2	4	4	2	
Micrococcus fulvum	0	0	1	2	0	0	0	0	0	0	1	0.5	
Fungi													
Aspergillusniger	1	2	0	0	2	4	1	2	0	0	4	2	>0.01*
Aspergillusfumigatus	0	0	1	2	1	2	0	0	2	4	4	2	
Mucorfuscus	0	0	0	0	1	2	0	0	1	2	2	1	

*The P value is not statistically significant at the 0.01 level.

Table 4. Percentage (%) of PMC Content of paracetamol syrups.

Product Code	PMC Content (%)	
	Minimum	Maximum
A	98.3	100.2
B	99.2	99.7
C	95.7	102.6
D	96.8	100.4
E	94.1	103.5

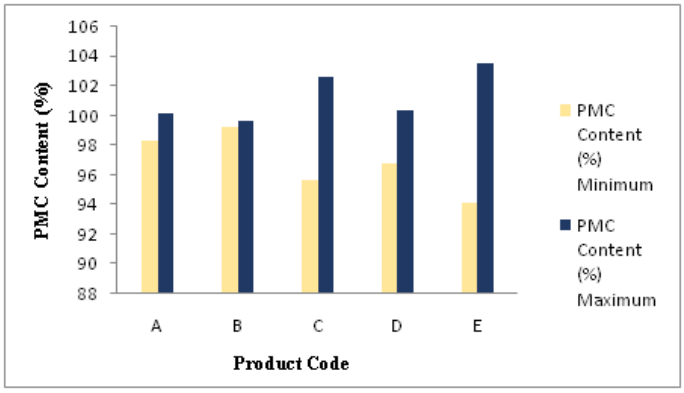

Figure 1. The Paracetamol (PCM) content results of analyzed analgesic syrup samples.

Discussion

Results obtained from this investigation have revealed that all the syrups complied with the official requirement for microbiological quality of syrups in the total viable aerobic count levels, according to the USP (2007) specification. This is in agreement with the work of other investigators [11-12].

From the findings made in this study, it could be inferred that very small levels of microbial contamination of the syrups in this investigation were observed. The low levels of microbial contamination in tested syrup samples could be due to the adoption of Current Good Manufacturing Practice (cGMP), effective preservative agents and adequate quality control program [13].

The lower total count of bacterial and fungal recorded in the syrups may be attributed to the sugar content of the syrups that provide high osmotic pressure that is inhibitory to many microorganisms. Moreover, syrups are usually filtered prior to bottling [21]. The USP (2007) recommends that *Salmonella* sp., *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus,* and *Candida albicans* as indicators of pathogenic microorganism contamination of syrups. All the samples of analyzed analgesic syrups were found to be free from the pathogenic microorganisms and passed the USP (2007) specifications. Similar observation was reported by Shaikh *et al.* (1988) [22].

Absence of coliform and pathogenic bacteria indicated that fecal contamination of water might not occur. Unhygienic environmental condition and improper handling of raw materials, ingredients, and products might be the cause of contamination [14].

Low water activity values usually inhibit the growth of bacteria such as members of the Enterobacteriaceae family, as well as aerobic and anaerobic spore formers, but allow the growth of certain vegetative microorganisms, such as Staphylococci and Micrococci, especially *S. aureus*, also the fungi such as *A. niger*, and *A. fumigates* which grow below a water activity of 0.86 [15].

The results of the present work showed that the most important isolated bacteria from non-sterile pharmaceutical syrups were *B. subtilis*, *M. fulvum*, and *S. epidermidis*, while the most important fungi were *A. niger*, *A. fumigatus*, and *P. notatum*. *Staphylococcus* sp. and *Bacillus* sp. might transmit from soil and hands of handler during the preparation of drugs. Their incidence does not always mean that the consumption of medicines are potentially being hazardous to users as not all the strain of *Staphylococcus* sp. can necessarily produce enterotoxin and higher infectious dose (105-106 CFU/mL) of *Bacillus* sp. is required [16].

Bacillus subtilis reported to be the most frequent in syrups and are widely distributed in the soil, dust, air, and water and because they are resistant to environmentally destructive factors. *S. epidermidis* was the most frequently isolated species from oral and topical medicaments. *Micrococcus* sp. was also isolated from liquid and solid drugs [27-28]. Some of the fungi isolated include species of *Aspergillus*, *Penicillium* and *Mucor* are possible allergic and toxin producers. *Aspergillus* sp. causes Aspergillosis while *Aspergillusflavus* produces aflatoxin that is carcinogenic [17].

The types of microorganisms isolated in this study suggest contamination from air, processing unit, during handling, and packaging materials.

In our study, the observation that the syrups were not cloudy is indicative of the absence of undesirable chemical and physical changes as well as the lack of visible microbial growth in the syrups.

In this study, the results showed that some the analgesic syrup samples appearance were red and pink with sweet taste, and this agreement with Ofonaike *et al.* (2007) [23], but remaining samples was bitter taste that not palatable for pediatric patient.

Liquid preparations for oral use may contain suitable excipients such as stabilizing, flavoring and sweetening agents and coloring matter, authorized by the competent authority (USP, 2007).

As per USP (2007) specifications the pH values in the analgesic syrup contained paracetamol from 3.8–6.1. From the results of Table 1, the pH value results were within the acceptable range according to USP (2007) standards.

The density results in the paracetamol syrup samples were 1.149–1.184 g/mL. The density of a substance is the ratio of its mass to its volume at 20°C. No pharmacopeia stipulates specification or limit for density value but leaves that to competent authority to authorize the density value.

As per USP (2007) specifications the percentage content of paracetamol in analgesic oral syrup ranges are from 90.0%–110.0% of the labeled amount in paracetamol syrups. Table-4 and Figure-1 indicate that products A, B, C, D & E showed values within the USP (2007)

specifications for a product to be passed. This is similar observation was reported by Oluseun *et al.* (2012) [24].

Conclusion

It can be concluded that the five different brands of paracetamol syrup sold in Bangladesh have passed the official requirement for microbiological quality of syrups. Also, the pH value and paracetamol content showed within the USP (2007) specifications for a product to be passed. The organoleptic test results had red and pink clear liquid with sweet taste in some analgesic syrups but remaining samples are bitter taste that not suitable for pediatric patients. It is therefore suggested that for better patient acceptability the taste of some paracetamol syrups should be improved and Good Manufacturing and Packaging Practice, proper treatment of water and air; personal hygiene improvement of the production personnel and pretreatment of natural raw materials be enforced and maintained.

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