PERFORMANCE EVALUATION OF DIFFERENT BRANDS OF SALBUTAMOL SULPHATE SYRUP

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Abstract: *Salbutamol sulphate is a short-acting β2-adrenergic receptor agonist used for the relief of bronchospasm in conditions such as asthma and chronic obstructive pulmonary disease (COPD). It is widely produced and marketed drug by many pharmaceutical companies in Bangladesh. Five different brands of salbutamol sulphate syrup manufactured by Bangladeshi pharmaceutical companies were collected from different retail pharmacy of Dhaka and Noakhali, Bangladesh. The performance evaluation (appearance, organoleptic properties, density, viscosity, pH, potency and microbial count) was carried out in order to find out whether they really comply with the required standards. The results of viscosities, density, and pH of the entire sample were within the official specification. The potency of the representative samples was determined by uv-spectrophotometric method, result of all brands were 96.07 % to 104.5% which is within the official limit. This study was also conducted to determine the microbiological quality of different brands. Microbiological analysis was carried out using spread plate technique on culture media. This study gives the idea about the overall quality of salbutamol sulphate syrup, manufactured by pharmaceutical companies of Bangladesh.*

Keywords: *Salbutamol sulphate, organoleptic properties, microbial assay, Bangladeshi medicines, performance evaluation, β2-Adrenergic receptor, β2-agonists.*

Introduction:

Salbutamol, otherwise called albuterol is a β2-adrenergic bronchodilator and marketed as various brand name and use as a prescription that opens up the medium and vast airways in the lungs[1].Salbutamol sulphate syrup mostly used for the treatment of asthma, exercise-induced bronchospasm, and chronic obstructive pulmonary disease (COPD) [2].Fine tremor of the hands is the most well-known side effect of Salbutamol due to stimulation of the peripheral skeletal β2-receptors, which may obstruct with exact manual work. Tension, restlessness and a rapid heartbeat may also occur[3].Apart from the serious side-effect which is inherent in the drug itself, such as teratogenicity, a medicinal product can become unsafe due to many other factors such as cross-contamination, contamination with pathogenic organisms, very high or low potency, wrong labeling, inadequate packaging and storage conditions. So a careful and rational evaluation should be needed[4].According to most pharmacopeias non-sterile dosage forms are not necessary to be sterile but are required to pass microbial tests for the absence of definite specified pathogens to certify their efficacy and safety. For two reasons the presence of the huge number of non-pathogenic microorganisms in pharmaceuticals is intolerable firstly these microorganisms can deteriorate active ingredients and can interfere with the desired activity of the product; and secondly, they can deliver some toxic metabolites that might be destructive to the consumer[5].In terms of both therapeutic efficacy and safety of the patients quality of medicine is an absolute necessity. World Health Organization claimed that the manufacturers must take charge of responsibility for the quality of the medicines that they are manufacturing[6].

The evaluation of drug product performance is important since bioavailability is related to the pharmacodynamics response and related adverse events. Therefore, performance tests relate the quality of a drug product to clinical safety and efficacy[7].As drug products are the lifesaving product so strength, quality, purity, and identity including the packaging, storage, and other requirements and specification should be according to the pharmacopeias standards to maintain it effectiveness. The expression "quality" has a relative significance. This is communicated by the ISO definition: "The totality of highlights and attributes of an item or administration that bear on its capacity to fulfill expressed or inferred needs"[8]. Each pharmaceuticals fabricating attempted to retain great quality to keep up its reputation and capture the competitive marketplace.

The overall numbers of brands of salbutamol sulphate that are registered in Bangladesh are currently estimated to be 97, whereas the aggregate number of salbutamol sulphate syrup is 37[9].

The real motivation behind this research is to find out the present status of the quality of the marketed Salbutamol Sulphate syrup preparations available in Bangladesh. This study will increase awareness among the peoples, health practitioners and drug control authority so that pharmaceutical manufacturers are forced by them to produce quality medicine and people may not waste their hard earning money by buying low quality product.

Materials and methods:

Instruments used in the study: Laboratory instruments such as Dissolution Test Apparatus USP (Minhua, RC-8), UV–Visible Spectrophotometer (T60U PG Instruments, England). Electronic Balance (Ohaus CP213 China), Oswald Viscometer (China), pH meter (Switzerland), Picnometer (China), Laminar air flow (Japan) were used in this study.

Reagents used in the study: The used chemicals were hydrochloric acid (CID 313), tryptone soy agar and sabouraud dextrose agar (SDA) and distilled water (CID: 962). All the reagents were analytical grade.

Collection of Sample: Samples from top, middle and lower category pharmaceutical companies were randomly selected and collected from retail pharmacies of Dhaka and Noakhali, Bangladesh. The samples were properly checked for their physical appearance, name of the manufacturer, batch number, date of manufacturing and expiry,

manufacturing license number, D.A.R /M.A number and maximum retail price at the time of purchase. Five different available brands of syrups of various manufacturers were purchased for the analytical studies. Ten batches of sample from each manufacturer were collected. The samples were then properly coded for analysis (SAL 01, SAL 02, SAL 03, SAL 04, and SAL 05).

Collection of reference standard: The working standard of Salbutamol Sulphate was obtained from GlaxoSmithKline Bangladesh Ltd. as gift sample for research work. The purity of the reference standard was 100.9%.

Physical analysis:

Organoleptic test of syrup: Analysis of the organoleptic properties of syrups were conducted by checking appearances, color, homogeneity, transparency, taste and the flavor by allowing syrup to expose to normal conditions at room temperature.

Density determination: Analysis of density of syrups were done by using picnometer by following the equation-

$$\rho = w/V$$

Where, ρ is the density, w is is the weight of sample and V is the volume.

Viscosity determination: The viscosity of the syrup was done by using Ostwald viscometer. It was calculated by using the following formula-

$$\eta_1 \rho_2 t_2 = \eta_2 \rho_1 t_1$$

Where, η_1 and η_2 are viscosity coefficients of the sample and water respectively, and ρ_1 and ρ_2 are the densities of sample and water, respectively.

pH measurement:

Suitable amount (30-40mL) of syrup was poured in a beaker. pH meter was immersed into the syrup. Then pH value was noted at room temperature.

Chemical analysis:

Preparation of standard curve:

To prepare a standard solution, 20 mg of salbutamol sulphate was weigh and placed in 100ml volumetric flask to prepare stock solution. Then a series of solution was prepared from stock solution by suitable dilution with 0.1N HCl with the concentration of 2µg/mL, 42µg/mL, 62µg/mL, 8µg/mL, 10µg/mL, 12µg/mL, 14µg/mL, 16µg/mL, 18µg/mL, and 20µg/mL. The absorbance were measured at 278 nm against blank for each solution and the average was calculated which described in Table-3.

Table 3: Absorbance of Standard at Different Concentration in 0.1N HCl at 278 nm

Concentration (µg/mL)	Absorbance	Average of the absorbance
2	0.029 0.030 0.028	0.029
4	0.039 0.038 0.040	0.039
6	0.151 0.150 0.152	0.151
8	0.173 0.172 0.174	0.173
10	0.188 0.187 0.189	0.188
12	0.242 0.241 0.243	0.242
14	0.125 0.124 0.126	0.125
16	0.140 0.139 0.141	0.140
18	0.205 0.204 0.206	0.205
20	0.168 0.167 0.169	0.168

Preparation of sample solution: 5 mL of syrup from each brand was placed in 100 mL volumetric flask and 60mL of 0.1N HCl was added to dilute and sonicated for 5 minutes and diluted to its final volume to make the concentration 10µg/mL. Then the absorbance was taken. The amount of salbutamol

sulphate was calculated according to the following equation.

$$\text{Potency} = \frac{Abs_{sam} \times Wt_{std} \times DF \times Wt/mL \times P}{Abs_{std} \times Wt_{sam}}$$

Where,

Abs_{sam} = Absorbance of Sample
Abs_{std} = Absorbance of Standard
Wt_{std} = Weight of Standard
Wt_{sam} = Weight of Sample
DF = Dilution Factor
Wt/mL = Weight per mL of syrup
P= Potency of standard

Microbial Assay of Syrup:

Preparation of Agar plates: Nutrient agar is a general purpose medium supporting growth of a wide range of non-fastidious organisms. 100 ml agar media was prepared and sterilize the media in an autoclave at 120º C, 15 psi for 20 min. Prepared the working area was suitable for the laminar air flow area. The plates were labeled with the type of media and poured into them. Swirled the hot media vigorously to mix. The media was cooled until it was just cool enough to handle, about 20–30 minutes. Swirled the media again to mix just before pouring; be careful not to incorporate bubbles. Then it was poured into plate until it covers the bottom, approximately 25ml. Plates will keep refrigerated for few minute.

Enumeration technique: The microorganism was enumerated by the Fawole and Oso methods and judged against the standard of microbiological specifications for qualifications of syrups[10].Using the spread plate technique, each sample of the syrup was vigorously shaken; 0.1 ml was withdrawn and diluted serially (10 fold dilution) in sterile normal saline. A quantity of 0.1 ml of the diluted sample was spread on the surface of tryptone Soy agar and sabouraud dextrose agar (SDA) plates. Spread plate technique was used to enumerate the microbial contaminant from the collected syrup samples. One milliliter from each sample was withdrawn aseptically and transferred into 9ml normal saline for serial dilution to 10-3. Diluted samples were thoroughly mixed for the proper dissolution of the drug. 0.1ml of each sample was then inoculated into different culture media plates by spread plate technique. The suspensions were reconstituted with sterile distilled water and were thoroughly mixed and cultured as for the syrups. The tryptone soy agar plates were incubated at 37^{o}C for 24 hours while the SDA plates were incubated at 25^{o}C for 7 days with daily observation[11].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 and suspension was calculated.

Isolation and Quantization: 0.1 ml of the each diluted sample was spread aseptically onto nutrient agar plate. Plates were then incubated for 24 hours at 37˚C. Bacterial colonies were counted manually and average number of colony forming unit (cfu) was determined for each ml of the syrup sample.

Identification of Isolated Micro-organisms: The sample of the syrups was placed on various selective media such as tryptone soy agar and sabouraud's dextrose agar (mould and yeasts). Growth characteristics, microscopy study and biochemical tests were used for the identification of isolates from the syrups. Samples were incubated for 24 hrs after which presence of growth was observed. The bacterial and fungal isolates were further identified using the standard media and biochemical analysis

Results and discussion:

Organoleptic evaluation of syrup: The organoleptic properties of five brands of salbutamol sulphate syrup were thoroughly analyzed and results are shown in the table 4.Organoleptic properties of different brands of salbutamol sulphate syrup can be different. But color, flavor, taste of all brands should be acceptable and syrup of all brands must be homogenous.

Table 4: Organoleptic evaluation of various brands of Salbutamol Sulphate syrup

Brands	Container	Color	Flavor	Taste	Clarity
SAL 01	White glass	Yellow	Strawberry	Sweet	Clear
SAL 02	Amber glass	Light green	Raspberry	Sweet	Clear
SAL 03	White plastic	Orange pink	Vanilla	Sweet	Clear
SAL 04	Amber glass	Colorless	Strawberry	Sweet	Clear
SAL 05	White plastic	Light green	Raspberry	Sweet	Clear

Physical analysis:

For the physical analysis density, viscosity, pH of the syrup was measured. The obtained result of these test were meet the specification of British Pharmacopoeia[12].

Result of density: It is seen from the above results (Table 5) that none of the samples exceeded the specification for density. Therefore, it can be said that all the studied samples complied with the USP specification for density of syrup (≤1.380g/ml).

Table 5: Density of various brands of Salbutamol Sulphate syrup

Sample code	No. of samples	Avg. density (g/ml)	Remarks[12]
SAL01	5	0.996	Complied
SAL 02	5	1.181	Complied
SAL 03	5	1.007	Complied
SAL04	5	1.054	Complied
SAL05	5	1.010	Complied

Result of viscosity: For Salbutamol Sulphate syrup not all brands re the same. Some are thin and flow easily, while others are thick and flow very slowly. In syrup, viscosity should be optimum. Because if it is more viscous then difficulties occur to withdrawal doses from the container and less viscous can increase the flow property of liquid. So it is difficult to measure the exact dose. It is seen from the above results of viscosity (Table 6) that none of the samples exceeded the specification for viscosity.

Table 6: Viscosity of various brands of Salbutamol Sulphate syrup

Sample code	No. of samples	Avg. viscosity (cp)	Remarks[12]
SAL 01	5	8.294	Complied
SAL 02	5	9.208	Complied
SAL 03	5	7.093	Complied
SAL O4	5	11.431	Complied
SAL05	5	10.038	Complied

Result of pH:

It is observed from the above results (Table 7) that none of the samples exceeded the specification for pH.

Table 7: pH of various brands of Salbutamol Sulphate syrup

Sample code	No. of samples	Average pH	Remarks[12]
SAL 01	5	4.35	Complied
SAL 02	5	4.12	Complied
SAL 03	5	4.02	Complied
SAL O4	5	4.26	Complied
SAL05	5	4.75	Complied

Chemical analysis:

Assay or potency testing was a chemical analysis that was performed by uv-spectrophotometric method.

Result of Potency: The ingredients of syrup samples exert the therapeutic effect. The deficient potency will result in less therapeutic response or even the product may be ineffective. From the above result (Table 8), it is observed that all brands of syrup meet the official specification of potency within 95-105%.

Table 8: Potency of various brands of Salbutamol Sulphate syrup

Sample code	Potency (mg/5ml)	Potency (%)	Remarks
SAL 01	2.089	104.5	Complied
SAL 02	2.056	102.8	Complied
SAL 03	1.922	96.07	Complied
SAL 04	1.948	97.43	Complied
SAL05	1.961	98.01	Complied

Result of microbial count: Acceptable limit of bacteria $\leq 10^3$cfu/ml and acceptable limit of fungi $\leq 10^2$cfu/ml for Salbutamol Sulphate syrup[13].From the above result (Table 9), it is

observed that all brands of syrup meet the specification of presence of microorganism.

Table9: Summary of Microbial Count of Salbutamol Sulphatesyrup

Sample	Total Aerobic bacterial count (cfu/ml)	Total Fungal count (cfu/ml)	Remarks
SAL 01	7×10^2	1×10^2	Complied
SAL 02	6×10^2	8×10^1	Complied
SAL 03	8×10^2	3×10^1	Complied
SAL 04	5×10^2	7×10^1	Complied
SAL 05	7×10^2	4×10^1	Complied

Conclusion:

Five brands' of Salbutamol Sulphate syrup was analyzed by using established method and calibrated apparatus in order to determine their quality. This study also helps to make comparison between different brands of Salbutamol Sulphate syrup manufactured by Bangladeshi pharmaceutical company. The present works reports comparative study of different brands of Salbutamol Sulphatesyrup. It can be concluded that most of these syrup have passed the official requirement for density, viscosity, pH, potency and microbial count. It helps people to find out quality product to treat asthma.Moreover this study will help both physician and consumers to select quality product also this work can provide some information for Drug Control Authority of Bangladesh to evaluate the overall quality status of Salbutamol Sulphate Syrup. Regulatory bodies should come forward and take necessary measures so that the manufacturers do not produce a sub-standard medicine which is threat for the patient.

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