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Research Article

Quality Assessment, Comparison, Detection and Determination of Various Adulterants in Different Milk Samples by Analytical Techniques

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ABSTRACT

Adulteration is defined as an act of intentionally debasing the quality of food offered for sale either by the admixture or substitution of inferior substances or by the removal of valuable ingredient. Milk is an essential commodity of life as it is a source of calcium and other essential nutrients required by the body. It is available in the market both locally and as a branded commodity. The study was carried out keeping in view the recently emerging concern of adulteration of milk with various illegal substances to increase its marketability. The aim of the study was to estimate different types of adulterants in various marketed milk brands and local samples collect from different areas of Hyderabad and to compare the quality of milk. Seven samples each of FSSAI approved brands were collected and subjected to various standard tests. The milk samples were estimated using different qualitative and quantitative tests and also compared the level of adulterants used in different branded milk samples by means of different chromatographic techniques. The concentrations of oxytocin, Diclofenac, and Antibiotic residues of samples 1 ,3 was found to be the closeness to the maximum residual limit whereas the samples like sample 2,4 and 5, milk were found to be more safe and the concentration of adulterants in these brands are comparatively less. on the other hand, the sample collected from local area is having the higher concentrations of oxytocin, Diclofenac, and Antibiotic residues than MRL.

Keywords: Adulteration; FSSAI, Quality of milk.

INTRODUCTION

Milk is the major source of strength for human beings as it contains a variety of nutrients like casein, lactose, vitamins and minerals. As a large number of people consume milk and milk products every day, there is a great need to monitor their quality and protect the common man against the deleterious effects of adulterants. Milk is a nutrient rich white liquid food; it is the primary source of nutrition[3]. As an agriculture product milk is extracted from farm animals, Adulteration of milk reduces the quality of milk and even make it hazardous. Most commonly the milk is adulterated with table sugars, starch, vegetable fats, urea, diclofenac sodium[4], antibiotic residues, oxytocin[1]. Milk is the major source of strength for human beings as it contains a variety of nutrients like casein, lactose, vitamins and minerals[8]. As a large number of people consume milk and milk products every day, there is a great need to monitor their quality and protect the common man against the deleterious effects of adulterants. According to the literature, Moore et al.2012 reported that milk powder is the second most likely food item being in the risk of adulteration after olive oil. Fischer, Schilter, Tritscher, & Stadler, 2011; Singh & Gandhi, 2015 reported that Adulterants in milk mainly include addition of vegetable protein, milk from different species, addition of whey and dilution with water which are known as economically motivated adulteration[10]. Apart from the above less harmful adulterants, some of the major adulterants in milk having severe adverse effects on human health are hormones, antibiotics, urea, formalin, detergents, ammonium sulphate, boric acid, caustic soda, benzoic acid, salicylic acid, hydrogen peroxide, sugars and melamine[11]. Buffalo milk is a rare jewel in the cheese making world. It is much higher in fat than cow's milk, but also lower in cholesterol. What this means is that buffalo milk is much richer, thicker, and creamier than cow's milk. But there are way more cheeses out there beyond those two. Buffalo milk is the most popular milk in Pakistan, India (which has 50 percent of the world's buffalo), and Italy. Water Buffalo create on average about 12-18 lb of milk a day (compared with 50-60 lb for a Holstein cow), so their output of milk is about the same as a goat but feeding requires about the same for a large cow. Milk adulteration is a very common food fraud and is posing a big social problem in today's world. Apart from the ethical and economical issue, it also creates health hazards[12].

MATERIALS AND METHODS

Materials

Table 1: Materials

Instruments	models
HPLC	WATERS, software: Empower, 2695 separation module, UV detector
UV/Vis spectroscopy	LABINDIA UV 3000, UV WIN SOFTWARE
pH meter	Adwa-AD 1020
Weighing machine	Sartorius-CPA, Afcoset ER-200A

Potentiometer	Eleco Digital Potentio Meter Model-118
Conductometer	Digisun electronics DI-909
Water bath	Sisco instrument
Sonicator	Soltec, spincotech instrument
Centrifuge	Remi

Estimation of Minor Adulterants in Milk

Water: The presence of water in milk was detected by addition of drops of milk sample on a polished slant surface. The drops of pure milk flow slowly leaving a white trial behind it whereas, milk adulterated with water flow immediately without a mark.

Starch: 5ml of milk sample was taken in a test tube. It was boiled, cooled and few drops of tincture of iodine or iodine solution was added to it. Starch was detected when the test tube content changed its colour to blue and absence of starch was noticed when there was no change in the colour.

Urea: A tea spoon of milk was taken in to a test tube and half tea spoon of soybean or arhar powder mix up was added to it. The test tubes were kept aside for 5 minutes. Later red litmus paper was dipped and it was removed after half a min. Urea was detected in the milk sample when the litmus paper changed its colour from red to blue.

Detergent: 5-10 ml of sample with equal amount of water was taken in a test tube and mixed up thoroughly. Presence of detergent was observed with the formation of lather whereas, absence of detergent was noticed with out the formation of lather.

Glucose/Invert Sugar: A diacertic strip was taken and dipped in milk sample for 30 sec to 1 min. Presence of glucose in milk sample was observed when the strip changed its colour and absence of glucose in milk sample was observed when the strip does not change its colour.

Vanaspathi: 3ml of milk sample was taken in to a test tube.10 drops of HCl was added and 1 tea spoon full of sugar was mixed to it. Mixture was examined after 5min. Vanaspati was detected, when the contents in the test tube changed its colour to red and absence of vanaspati was noticed, when there was no change in the colour of contents of the test tube.

Formalin: Formalin enhances the life of milk. So, it was added for preservative purpose. Use of formalin shown variable results regarding its ability to preserve samples. Various reasons, such as use of substandard formalin, storage period, storage temperature, nature of the sample preserved, had been proposed to explain this variability. The preservative had been shown to affect various physico-chemical properties as well as the fat, protein, lactose and total solids content during storage. This had created a problem and brought industry in direct conflict with the regulatory agencies. Many research workers had studied the compositional changes in milk samples as affected by formalin preservation.

Salt: 5ml of silver nitrate reagent was taken in a test tube. To this 2-3 drops of potassium dichromate reagent and 1 ml of milk were added and mixed thoroughly. If the contents of the test tube turned to yellow then salt was observed in the milk sample. If it was turned to chocolate or reddish-brown colour then sample was considered to be free from salt.

Hydrogen Peroxide: 5ml of milk sample was taken in a test tube and 3 drops of paraphenylenediamine was added to it and shaken well. Change in colour of milk to blue conforms that the milk was adulterated with Hydrogen peroxide.

Sugar: 3 ml of milk sample was taken in a test tube. To this 2 ml of HCl and 50mg of resorcinol was added and heated. Sugar was detected, when the contents in the test tube changed its colour to red and absence of it was declared when there was no change in the colour.

Boric Acid: 3 ml of milk sample was taken in to a test tube. 20 drops of HCl were added and shaken. A yellow paper strip was dipped in to it. It was removed the same after 1 min. Boric acid was detected when there was a change in the colour from yellow to red which was followed by a change from red to green by addition of 1 drop of ammonia solution.

Yellow paper strips of filter paper were prepared in an aqueous solution of the turmeric which were later dried it up.

Removal of Fat: The lactometer reading will go above 26.

Total Solids in Milk: To determine the total solids content of milk, 5g of milk sample was placed in pre- dried and tarred duplicate crucibles, which were labeled with a pencil. Then after the milk sample was evaporated to dryness on steam bath, the sample was kept at 102^{o} C in a hot air oven (model 101- 1A Tianjin Taisite Inst. Co. Ltd) for 3 hours. The dried samples were taken out from the oven, cooled to room temperature and then weighed.

(Richardson, 1985). Total solids (TS percent) = (Weight of dried sample) ×100 sample weight

Titratable Acidity in Milk: The Titratable acidity (TA) of both yoghurt and milk were determined using the method described by (Marth 1978). Sample (9 g) was weighed in 100 ml wide mouth flask. 20 ml of fresh distilled water (in case of yoghurt only) was added and then titrated against 0.1 N NaOH after addition of 3-5 drops of 1 percent phenolphthalein solution until persistent (30 sec.) faint pink color was observed. The Titratable acidity was expressed as percentage of lactic acid using the following formula Lacticacid(percent) = ml N alkali ×0.009 ml of sample used × 100.

Estimation of major adulterants in milk

Estimation of oxytocin by using RP-HPLC Method

Preparation of mobile phase: Acetonitrile:0.03M phosphate buffer, pH3.5(Dil. Ortho phosphoric acid)

Preparation of Standard solution: Accurately weighed quantities of oxytocin (10 mg) was taken in a 10ml volumetric flask and dissolved in HPLC grade water followed by dilution up to the mark with HPLC grade water (1000 µg / ml).

Preparation of milk Sample: Concentration of milk was prepared by 1 ml of sample solution with 1 ml of ice-cold solution of acetone. This solution was mixed and centrifuged at 3500 rpm in the centrifugal apparatus. After centrifugation process, the acetone layer was taken and mixed with 1 ml of petroleum ether. This solution was mixed and kept for 5 minutes. After 5 minutes, the ether layer was discarded and the lowerlayer was evaporated to dryness. To this 0.2 ml buffer solution was added and filtered. The filtered solution was injected[1.]

Estimation of diclofenac sodium by using RP-HPLC Method

Preparation of mobile phase: ACN: Phosphate buffer pH 7.0 (50:50v/v) Preparation of standard solution: Weighed accurately 100mg of diclofenac sodium was taken in 100ml of volumetric flask. To this 25ml of mobile phase was added .

Preparation of milk sample: 4 test tubes were taken and each test tube consisted of 4 ml of methanol and 1 ml of milk sample and mixed well with the help of shaker system so as to filtrate each sample with Whitman filter paper. Then the samples were ready for analyzing with HPLC Peak. Now from the filtrate milk sample, 20µL Sample was taken and injected into the H.P.L.C. Then H.P.L.C Report was observed. The drug value was estimated by statistical formula. The sample (liquid 20 % v/v, solid 20 %w/v) preparation was performed with a mixture of Water-Methanol (30%:70% v/v) which was shaken and followed by ultra-filtration. Prepared samples were injected in to H.P.L.C for the estimation of quantity of Diclofenac sodium[4].

Estimation of antibiotic residues by using RP-HPLC

Preparation of buffer (pH 4.0): weighed quantities of 5.04 gm Disodium hydrogen phosphate and 3.01 gm potassium dihydrogen phosphate were taken and dissolved in sufficient water so as to produce 1000 ml and the pH of solution was adjusted with glacial acetic acid.

Preparation of mobile phase: A required volume of degassed mixture of buffer and acetonitrile in the ratio of 55:45 v/v was prepared.

Preparation of diluent: Mobile phase was used as diluent.

Preparation of standard solution: Accurately weighed and transferred 100 mg of ciprofloxacin working standard into a 100 ml clean, dry volumetric flask. (1000µg/ml)

• 1mL of standard stock solution was taken in to a 100 mL volumetric flask and diluted with mobile phase as diluent up to the mark and mixed well. (10 µg/ml).

• Further 2,4,6,8,10 ml of standard stock solutions were taken in 100ml of volumetric flasks and diluted with mobile phase up to the mark as mixed well. Results were in 0.2, 0.4, 0.6, 0.8, 1 µg/ml concentrations respectively[5].

Preparation of sample solution:

Liquid phase extraction:

• 4gm of weighed sample was taken in a clean beaker. The homogenization was done with the addition of 10ml phosphate buffer to the sample (pH was adjusted to 6.5) and mixed thoroughly.

• Protein content of these samples were precipitated with the addition of 2ml trichloroacetic acid (30%).

• Then these samples were taken in to properly cleaned and sterilized centrifuge tubes for centrifugation. The centrifugation was performed at 7000rpm for 15 min.

• Then filtration of the supernatant was performed with the help of whatsmann filter paper and funnel, then these filtrates were extracted with an equal volume of diethyl ether to perform defatting.

• Then cleaned and sterilized separating funnels were used for the separation of mixture from each other. Only upper oily layer was discarded and bottom layer was collected.

• This extraction of supernatant was repeated twice with diethyl ether, and then these extracts were evaporated until dryness.

• The dried extract was reconstituted with 2ml mobile phase as diluents.

• Then filtered with 0.45µ membrane filter.

RESULTS AND DISCUSSION

Estimation of minor adulterants in milk

By this quantitative analysis we came to know that the local milk sample contain adulterants such as (water, glucose, detergent).

By this quantitative analysis we came to know that:

All local milk samples contain water as adulterant. Glucose was present in all samples except in sample1.Titerable acidity was greater in sample 1 and chloride content was greater in sample 3. Adulterants such as starch, urea, vanaspati, formalin, salt, hydrogen peroxide, sugar, boric acid were absent in all milk samples.

Table 2: Minor adulterants in milk

S.No	Adulterant	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
1	Water	✓	✓	✓	✓	✓
2	Starch	-	-	-	-	-
3	Urea	-	-	-	-	-
4	Detergent	✓	-	✓	✓	-
5	Glucose/invert sugar	-	✓	✓	✓	✓
6	Vanaspati	-	-	-	-	-
7	Formalin	-	-	-	-	-
8	Salt	-	-	-	-	-
9	Hydrogen peroxide	-	-	-	-	-
10	Sugar	-	-	-	-	-
11	Boric acid	-	-	-	-	-
12	Titratable acidity	50%	32.5%	41%	40%	42%
13	Chloride content	2.2%	1.6%	6.66%	1.2%	2%

Estimation of major adulterants in milk

Estimation of oxytocin by using RP-HPLC Method

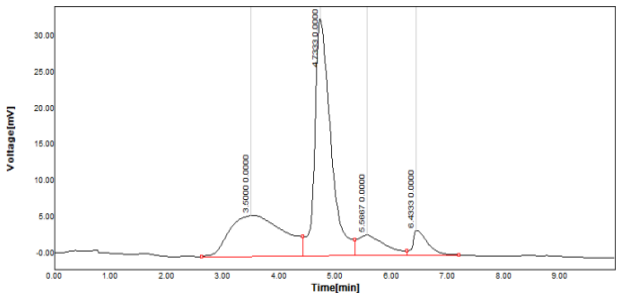

Figure 1: Chromatogram of oxytocin

Comparative study:

Table 3: Comparative studies of oxytocin

Milk brand	Oxytocin Concentration µg/mL	MRL µg/day/person
Sample 1	1.9	2.3-2.4
Sample 2	1.1	2.3-2.4
Sample 3	1.8	2.3-2.4
Sample 4	0.9	2.3-2.4
Sample 5	1.2	2.3-2.4

Oxytocin Concentration was found to be higher in sample 1(1.9) and lower in sample 4(0.9). Oxytocin Concentration of all milk samples within acceptance limit 2.3-2.4 µg/ml. The sample 4 was considered to be better among all other samples as, it consisted of lower amount of oxytocin.

Estimation of diclofenac sodium by using RP-HPLC method

Diclofenac sodium Concentration was found to be higher in sample 1(0.092) and lower in sample 4(0.012). Diclofenac sodium Concentration of all milk samples within acceptance limit 0.1 µg/ml. The sample 4 was considered to be better among all other samples as, it consisted of lower amount of Diclofenac sodium.`

Table 4: Comparative studies of diclofenac sodium

Milk brand	Diclofenac sodium Concentration (µg/ml)	MRL µg/ml
Sample 1	0.092	0.1
Sample 2	0.041	0.1
Sample 3	0.065	0.1
Sample 4	0.012	0.1
Sample 5	0.034	0.1

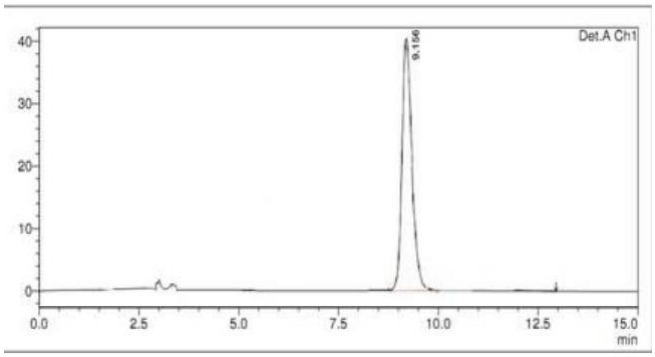

Figure 2: Chromatogram of diclofenac sodium

Estimation of antibiotic residues by using RP-HPLC

Ciprofloxacin Concentration was found to be higher in sample 1(0.09) and lower in sample 4(0.025). Ciprofloxacin Concentration of all milk samples within acceptance limit 0.1µg/ml. The sample 4 was considered to be better among all other samples as, it consisted of lower amount of Ciprofloxacin.`

Table 5: Comparative studies of antibiotic residue

Milk brand	Ciprofloxacin Concentration (ppm/kg)	MRL (ppm/kg)
Sample 1	0.09	0.1
Sample 2	0.051	0.1
Sample 3	0.074	0.1
Sample 4	0.025	0.1
Sample 5	0.045	0.1

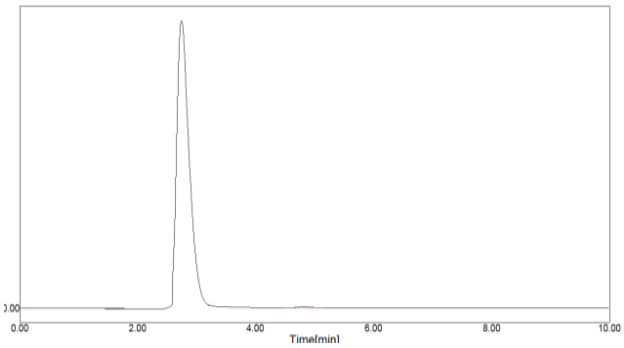

Figure 3: Chromatogram of optimizes method of antibiotic residue

The analytical peak was good. The plate count, tailing factor, and the resolution was found to be satisfactory.

CONCLUSION

At the end of the project, we established various qualitative and quantitative methods of identification of adulteration in milk samples and to estimate the level of adulteration in each sample. Assessment of major adulterers such as residues of antibiotics, hormones and diclofenac sodium were done by different methods in various brand milk samples and also compared the level of impurities in different brands of milk samples with the help of different chromatographic methods. Concentrations of Oxytocin, diclofenac, and residues of antibiotics of sample 5 were close to the maximum residue limit, while samples such as sample 2, sample 4 and sample 5 were found to be safer and the concentration of impurities in these brands are comparatively less.

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