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SPIKED FORCE DEGRADATION ASSAY METHOD EVALUATION OF CAMEL MILK IN DRY POWDER DOSAGE FORM

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ABSTRACT

Following experimental trials that may be summarised, the separation has to be achieved at optimal circumstances. Static phases such as Hypersil BDS C18 were most suited because they provided well-resolved peak shapes with high resolution and excellent sensitivity. Keeping the flow at 1 mL min-1 ensures high resolution. Camel Milk Dry powder was tested for PDA detector response and the wavelength with the maximum sensitivity was determined to be 240 nm. In order to separate Camel Milk Dry powder, a 40:60 percent v/v combination of ethanol and methanol was employed as the mobile phase, moving at 1mL/min. The temperature of the column was kept constant at room temperature.

KEYWORDS: Camel Milk Dry powder, Ethanol and Methanol.

INTRODUCTION

Camel Milk could be a profitable supply of crude fabric for numerous dairy powder makers, and it is getting to be more prevalent. The lion's share of analysts have concentrated their think about on the make of Camel Milk powder, as well as its capacity solidness and capacities. Other Milk powders, such as camel Milk powder, are, on the other hand, a source of extraordinary instability. In truth, since of its medicinal and nutritious qualities, camel Milk is the foremost regularly eaten Milk in dry and semi-arid ranges around the world.

Experimental

Methodology

Preparation of Standard Stock Solution

Preparation of Diluent

Following experimental trials that may be summarised, the separation has to be achieved at optimal circumstances. Static phases such as Hypersil BDS C18 were most suited because they provided well-resolved peak shapes with high resolution and excellent sensitivity. Keeping the flow at 1 mL min-1 ensures high resolution. Camel Milk Dry powder was tested for PDA detector response and the wavelength with the maximum sensitivity was determined to be 240 nm.

In order to separate Camel Milk Dry powder, a 40:60 percent v/v combination of ethanol and methanol was employed as the mobile phase, moving at 1mL/min. The temperature of the column was kept constant at room temperature.

Preparation of internal standard solution

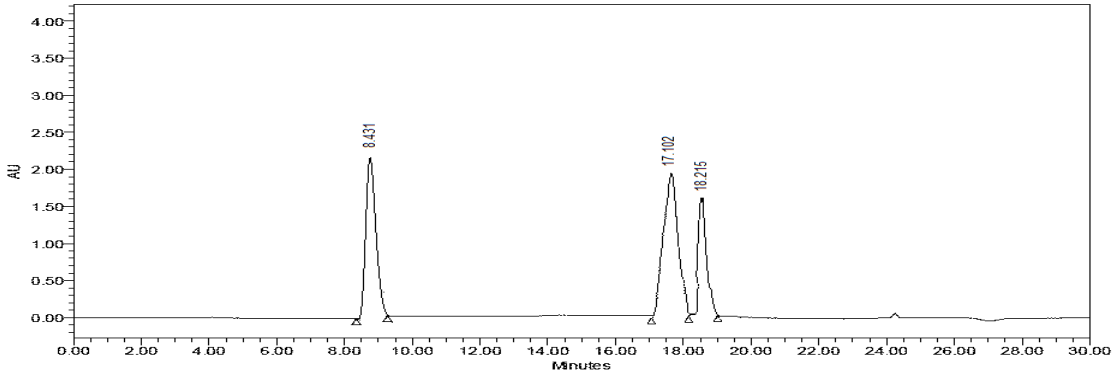
Weighed accurately about 10 mg of Camel Milk Dry powder working standard and transfer to 100 ml volumetric flask, add 50 ml of mobile phase and sonicate to dissolve it completely and then volume was made up to the mark with mobile phase to get 100 µg/ml of standard stock solution of working standard. Then it was ultrasonicated for 10 minutes and filtered through 0.20 µ membrane filter.

Preparation of Camel Milk Dry powder standard solution

Weighed accurately about 10 mg of Camel Milk Dry powder and transfer to 100 ml volumetric flask, add 50 ml of mobile phase and sonicate to dissolve it completely and then volume was made up to the mark with mobile phase to get 100 µg/ml of standard stock solution of working standard. Then it was ultrasonicated for 10 minutes and filtered through 0.20 µ membrane filter.

Camel Milk Dry powder	
System	UPLC
Stationary Phase	C18 column
"Mobile Phase"	"Ethanol and Methanol in the ratio of 40:60%v/v"
Diluents	Acetonitrile
Injection volume	5µl
Temperature	Ambient
Flow rate	1.0 ml/min
UV detection	240nm
Retention Time	Lactoferrin – 17.102 mins; 18.215 mins; Casein – 8.431 mins
Inference	"Satisfactory separation of the drugs was achieved with good resolution and minimal tailing."

Camel Milk Dry powder in UPLC System



Chromatogram of standard preparation of Camel Milk Dry powder ("Ethanol and Methanol in the ratio of 40:60%v/v")

➢ **Validation of Related Substance Studies for Camel Milk**

Accuracy Procedure: The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness. The accuracy of the method was evaluated in triplicate at three concentration levels, 50%, 100% and 150% of the target test concentration. The percentages of recoveries were calculated.

"***Accuracy 50%:*** "From the prepared stock solution 0.2 mL solution was transferred to a 10 mL volumetric flask and diluted to the mark with mobile phase to obtain a working sample solution of Camel Milk (2 µg/mL)."

"***Accuracy 100%:*** From the prepared stock solution 0.4 mL solution was transferred to a 10 mL volumetric flask and diluted to the mark with mobile phase to obtain a working sample solution of Camel Milk (4 µg/mL)."

"***Accuracy 150%:*** From the prepared stock solution 0.6 mL solution was transferred to a 10 mL volumetric flask and diluted to the mark with mobile phase to obtain a working sample solution of Camel Milk (6 µg/mL)."

Camel Milk						
Level %	**Amount added (µg/ml)**	**Amount found (µg/ml)**	**% Recovery**	**Mean recovery (%)**	**Std.Dev**	**% RSD**
50	02.14	02.13	99.07	99.47%	0.27005	0.28%
100	04.15	04.04	99.55			
150	06.16	06.15	99.84			

Accuracy

System Precision

The parameters, retention time (RT), theoretical plates (N), tailing factor (T), peak asymmetry (As) and repeatability were evaluated at a concentration of 4µg/mL (Camel Milk).

Parameters	**Camel Milk**
Retention time (min) ± % RSD	17.387 ± 0.05 ; 18.367 ± 0.05
Theoretical plates ± % RSD	4833.38 ± 0.50; 6507.98 ± 0.50
Asymmetry ± % RSD	1.05 ± 0.05; 1.05 ± 0.05
Repeatability (% RSD)	0.45; 0.48

System Precision

Method Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Acceptance Criteria: %RSD is nmt 2%

""**Procedure:** Precision was investigated using the sample preparation procedure for six consecutive replicates of sample of concentration 4 µg/mL for Camel Milk."

Replicate	***Camel Milk***		
S. No.	**Concentration Taken (µg/ml)**	**Area**	**%LC**
1	04.00	45746	99.94%
2		45734	99.95%
3		45766	99.94%
4		45677	99.85%
5		45698	99.82%
6		45753	99.78%
% RSD			0.09%
Standard weight			4mg
Standard potency			99.60%

Method Precision

Linearity

Camel Milk		
Linearity level	**Concentration in µg/mL**	**Area**
1	2 µg/mL	45768
2	4 µg/mL	50344
3	6 µg/mL	54923
4	8 µg/mL	59494
5	10 µg/mL	64075
Correlation co-efficient	0.9996	
Slope	1141.25	
Intercept	40250.1	

Linearity

Robustness

Robustness Studies			
Parameter	**Value**	**Peak Area**	**% RSD**
Flow Rate	**Low**	45782	0.11%
	Actual	45766	
	Plus	45785	
Temperature	**Low**	45782	0.67%
	Actual	45774	
	Plus	45775	
Wavelength	**Low**	45768	0.07%
	Actual	45784	
	Plus	45786	

Robustness

Ruggedness

"**Intraday precision (Repeatability**): Intraday Precision was performed and % RSD for Camel Milk was 0.11%."

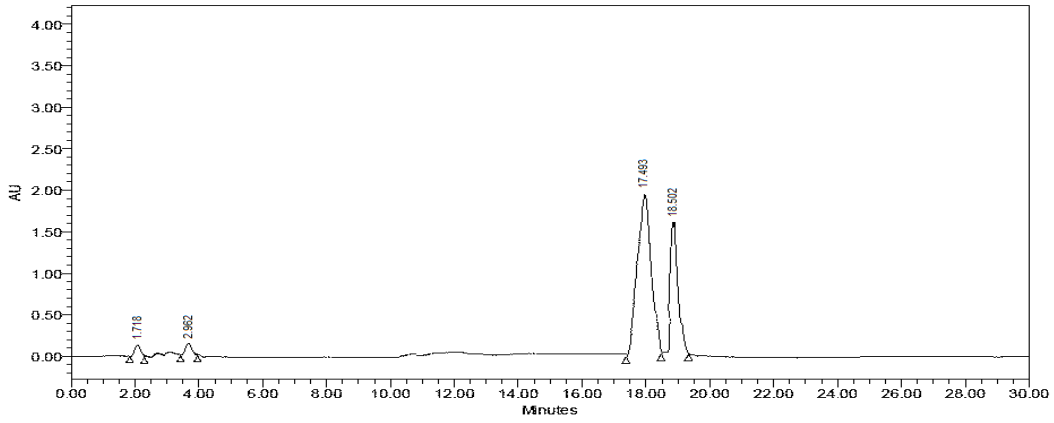
"**Inter day precision:** Inter day precision was performed with 24 hrs time lag and the %RSD Obtained for Camel Milk was 0.15%."

Camel Milk			
Ruggedness			
Parameter	**Peak Area**	**% RSD**	**%LC**
Intraday precision	45797	0.46%	98.94%
	45808		99.12%
	45795		99.77%
Inter day precision	45853	0.47%	98.94%
	45815		99.08%
	45836		99.83%
Instrument:1 Acquity UPLC Waters,2695H	45824	0.42%	99.54%
	45786		99.67%
	45797		98.93%
Instrument:2 Agilent Technologies, 1290	45838	0.41%	99.52%
	45794		99.64%
	45796		98.95%
Average			**99.23%**
Std.Dev			**0.3688**
%RSD			**0.37%**

Ruggedness

ASSAY

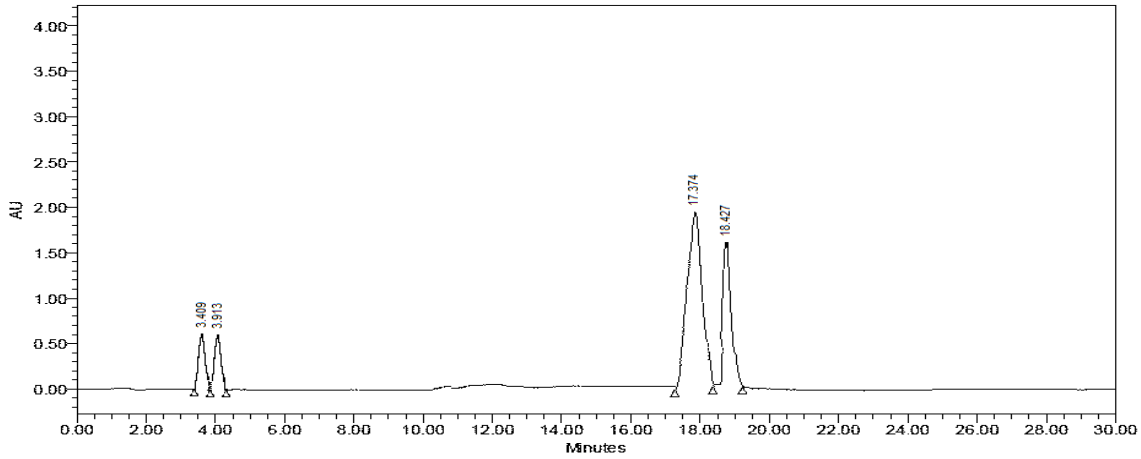
a. Acidic Degradation: "An accurate 10 ml of pure product sample solution was transferred to a clean and dry round bottom flask (RBF). 30 ml of 0.1 N HCl was added to it. It was refluxed in a water bath at 60°C for 6 hours. Product became soluble after reflux which was insoluble initially. Allowed to cool at room temperature. The sample was then neutralized using 2N NaOH solution and final volume of the sample was made up to 100ml with water to prepare 100ppm solution. It was injected into the UPLC system against a blank of mobile phase after optimizing the mobile phase composition, chromatogram was recorded and shown in Chromatogram."



Acidic Degradation

b. Basic Degradation

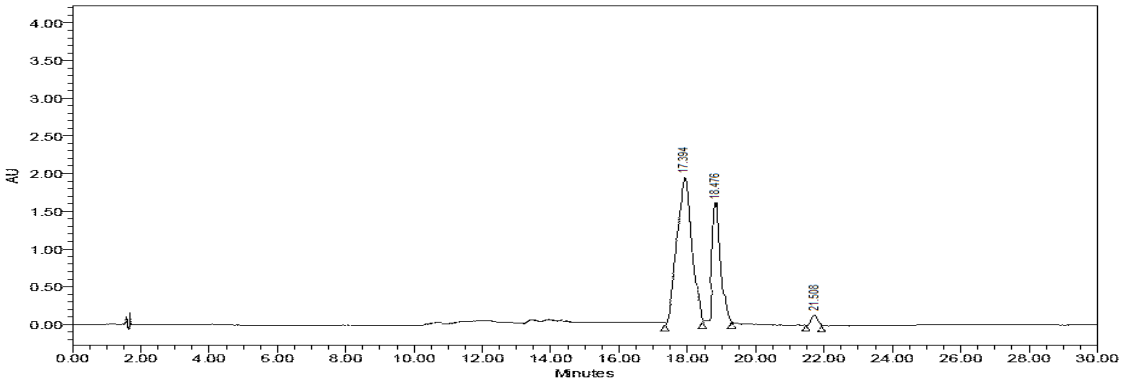
"An accurate 10 ml of pure drug sample solution was transferred to a clean and dry RBF. 30 ml of 0.1N NaOH was added to it. It was refluxed in a water bath at 60°C for 6 hours. Drug became soluble after reflux which was insoluble initially. It was allowed to cool at room temperature. The sample was then neutralized using 2N HCl solution and final volume of the sample was made up to 100ml with water to prepare 100ppm solution. It was injected into the UPLC system against a blank of mobile phase after optimizing the mobile phase composition, chromatogram was recorded and shown in Chromatogram."



Basic Degradation

c. Wet heat degradation

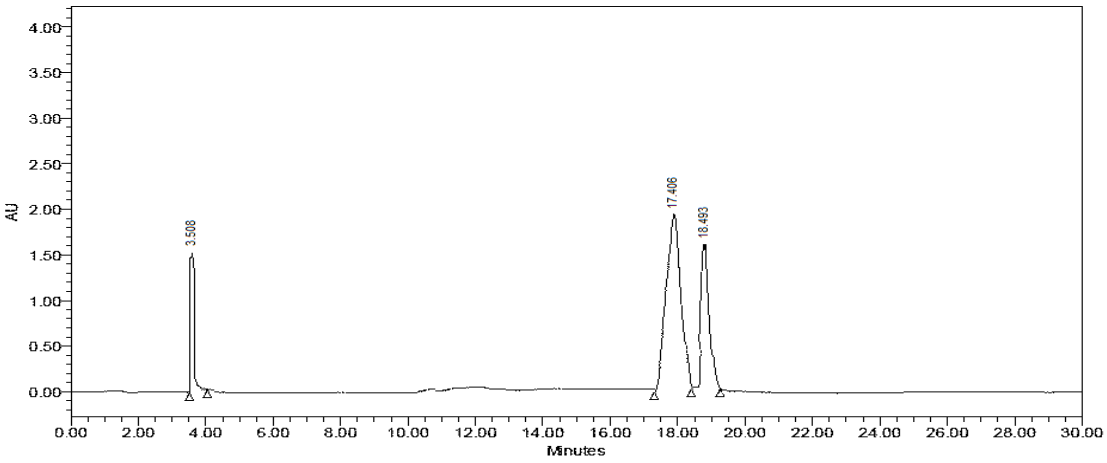
"Accurate 10 ml of pure drug sample was transferred to a clean and dry RBF. 30 ml of HPLC grade water was added to it. Then, it was refluxed in a water bath at 60°C for 6 hours uninterruptedly. After the completion of reflux, the drug became soluble and the mixture of drug and water was allowed to cool at room temperature. Final volume was made up to 100 ml with HPLC grade water to prepare 100 ppm solution. It was injected into the UPLC system against a blank of mobile phase after optimizing the mobile phase composition, chromatogram was recorded and shown in Chromatogram."



Wet heat Degradation

d. Oxidation with (3%) H_2O_2

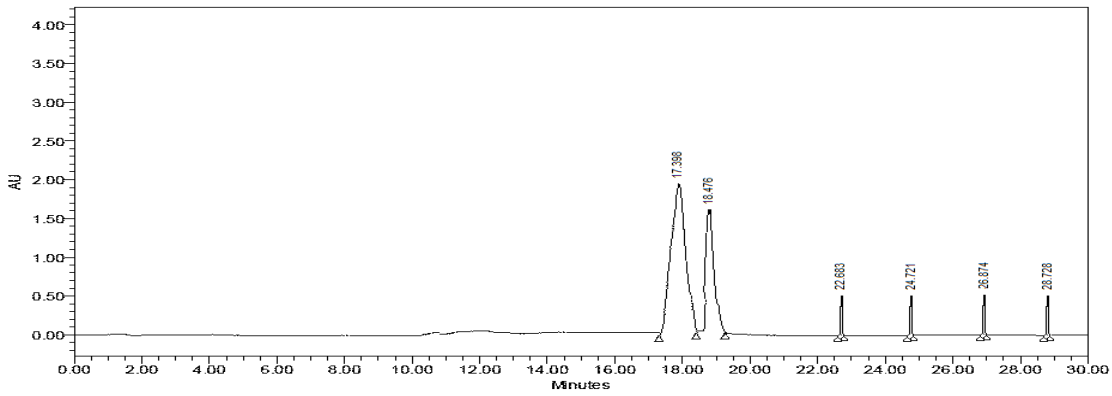
"Approximately 10 ml of pure drug sample was transferred in a clean and dry 100 ml volumetric flask. 30 ml of 3% H_2O_2 and a little methanol was added to it to make it soluble and then kept as such in dark for 24 hours. Final volume was made up to 100 ml using water to prepare 100 ppm solution. The above sample was injected into the UPLC system. The chromatogram was recorded and shown in Chromatogram."



Oxidation Degradation

Photolytic degradation

The photochemical stability of the drug was also studied by exposing the drug solution (4ml) to sunlight for 6 h. Twenty microlitres of the resultant solutions were injected onto column and the chromatograms were run as described.



Photolytic degradation

Nature of Stress	Degradation condition	Time(h)	Number of degradation products
Acidic	60°C	6	2
Basic	60°C	6	2
Oxidative	RT	6	1
Wet Heat	105°C	6	1
Photolytic	AT	6	4

Force Degradation

Calculation formula

$$\%\ Assay = \frac{AT}{AS} \times \frac{W1}{100} \times \frac{1}{25} \times \frac{100}{W2} \times \frac{25}{1} \times \frac{AW}{LC} \times P$$

"Whereas,"

"AT = Average area of test preparation, 26139"

"AS = Average area of standard preparation, 28358"

"W1 = Weight taken of reference standard (µg), 04.15"

"W2 = Weight taken of test sample (µg), 04.25"

"AW = Average weight of sample (µg), 3057"

"LC = Label claim (µg), 3000"

"P = Potency of reference standard (%), 99.98%"

$$\%\ Assay = \frac{AT}{AS} \times \frac{W1}{100} \times \frac{1}{25} \times \frac{100}{W2} \times \frac{25}{1} \times \frac{AW}{LC} \times P$$

Acidic Degradation

$$\%\ Assay = \frac{24721}{28358} \times \frac{04.15}{100} \times \frac{1}{25} \times \frac{100}{04.25} \times \frac{25}{1} \times \textbf{Error!} \times 99.98 = 86.73\%$$

Basic Degradation

$$\%\ Assay = \frac{23581}{28358} \times \frac{04.15}{100} \times \frac{1}{25} \times \frac{100}{04.25} \times \frac{25}{1} \times \textbf{Error!} \times 99.98 = 82.72\%$$

Oxidative Degradation

$$\%\ Assay = \frac{24357}{28358} \times \frac{04.15}{100} \times \frac{1}{25} \times \frac{100}{04.25} \times \frac{25}{1} \times \textbf{Error!} \times 99.98 = 85.44\%$$

Wet Heat

$$\%\ Assay = \frac{25832}{28358} \times \frac{04.15}{100} \times \frac{1}{25} \times \frac{100}{04.25} \times \frac{25}{1} \times \textbf{Error!} \times 99.98 = 90.62\%$$

Photolytic

$$\%\ Assay = \frac{25874}{28396} \times \frac{04.15}{100} \times \frac{1}{25} \times \frac{100}{04.25} \times \frac{25}{1} \times \textbf{Error!} \times 99.98 = 90.74\%$$

CONCLUSION

A short, specific, accurate, exact, and delicate procedure was brought up to determine the quantitative quantities of process-related pollutants and Camel Milk corruption items in pharmaceutical formulations. During a stretch inquiry, the debasement items of Camel Milk could be successfully segregated from the Camel Milk as well as its impurities, and the mass equalizations were shown to be adequate under all push conditions, demonstrating the method's ability to identify soundness. When it came to understanding recommendations, this method's specificity, linearity, restriction on where to look and how much it weighs were all validated by the Universal Conference on Understanding Guidelines (UCUN).

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