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EVALUATION AND VALIDATION OF A UPLC METHOD FOR ESTIMATION OF CAMEL MILK IN MARKETED (ADVIK) DRY POWDER DOSAGE FORM

Dr. Osman Ahmed*[1], Mohammed Akthar Sulthana[1], Ashraf Unnisa[1], Meher Afrin[1] and Dr. Anas Rasheed[2]

[1]Department of Pharmaceutical Analysis, Deccan School of Pharmacy, Hyderabad.
[2]CSO, Gaelib Medications Private Limited, Hyderabad.

***Corresponding Author: Dr. Osman Ahmed**
Department of Pharmaceutical Analysis, Deccan School of Pharmacy, Hyderabad.

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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

To obtain the separation under optimal circumstances after experimental trials that can be summed up, it is necessary to conduct experiments. Using a stationary phase like Hypersil BDS C18 was the best option since it generated well-resolved, high-resolution symmetrical peaks with excellent sensitivity and resolution. The flow rate of 1 mL min-1 was kept constant, and the results indicate high resolution. 240 nm wavelength showed the maximum sensitivity in a study of Camel Milk Dry powder's PDA detector response.

An acceptable mobile phase for the separation of Camel Milk Dry powder was discovered to be a combination of two solutions: ethanol and methanol, in the ratio of 40:60 percent v/v. A constant room temperature was used for the column's operation.

Preparation of internal standard solution

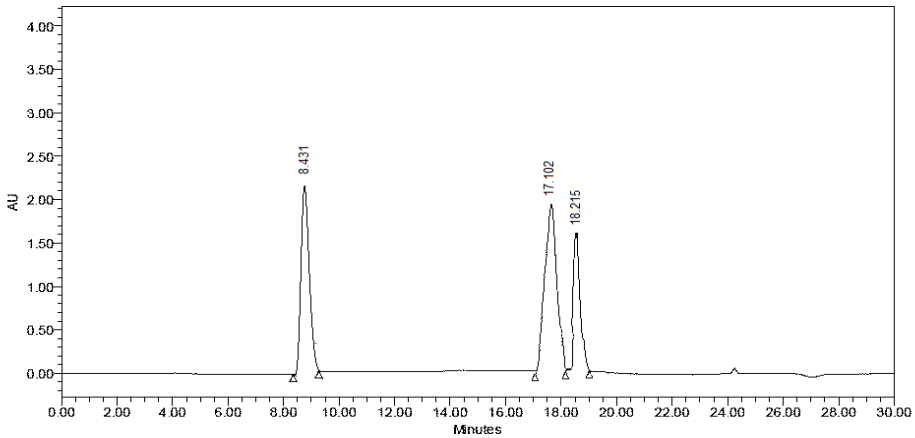
Weighed accurately about 10 mg of Camel Milk Dry powder working standard and transfer to 100 ml volumetric flask, add 50 ml of mobile phase and sonicate to dissolve it completely and then volume was made up to the mark with mobile phase to get 100 µg/ml of standard stock solution of working standard. 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 in UPLC System

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."



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)."

Accuracy

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			

System Precision

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

System Precision

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

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."

Method Precision

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%

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%."

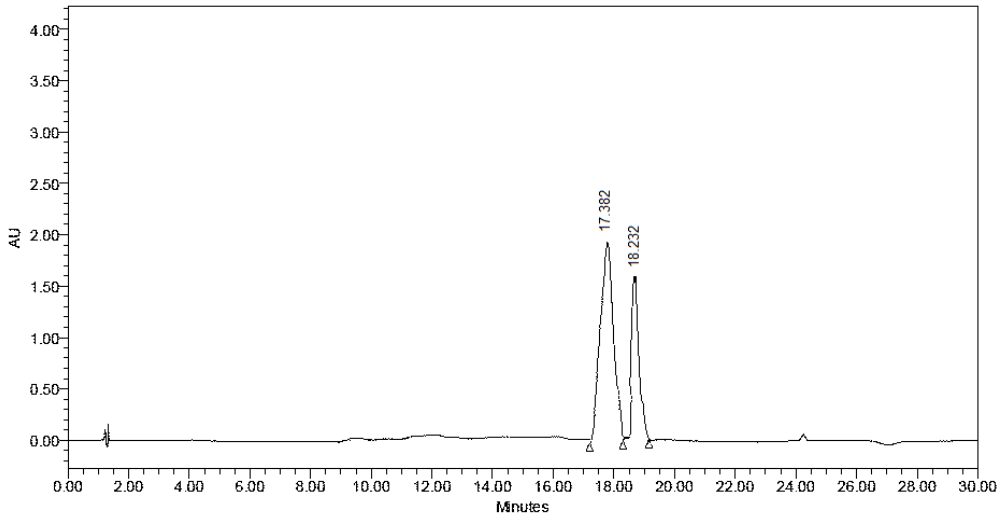
Ruggedness

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%**

ASSAY

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



Standard

$\%\ Assay = \frac{26139}{28358} \times \frac{04.15}{100} \times \frac{1}{25} \times \frac{100}{04.25} \times \frac{25}{1} \times$ **Error!** $\times$ 99.98 = 91.68%

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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