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EVALUATION AND VALIDATION OF A UPLC METHOD FOR ESTIMATION OF CAMEL MILK IN BULK 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. It displays excellent resolution when the flow rate is kept at 0.5 mL min-1. Camel Milk Dry powder was tested for PDA detector response and the wavelength with the maximum sensitivity was determined to be 230 nm. Camel Milk Dry powder could be separated using a mobile phase consisting of a 30:70% v/v combination of chloroform and methanol, which was applied at a rate of 0.5mL/min. The temperature of the column was kept constant at room temperature.

KEYWORDS: Camel Milk Dry powder, Chloroform 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

In order to achieve the separation under the optimized conditions after experimental trials that can be summarized. Stationary phase like Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 µm) column was most suitable one, since it produced symmetrical peaks with high resolution and a very good sensitivity and with good resolution. The flow rate was maintained 0.5 mL min-1 shows good resolution. The PDA detector response of Camel Milk Dry powder was studied and the best wavelength was found to be 230 nm showing highest sensitivity.

The mixture of two solutions Chloroform and Methanol in the ratio of 30:70%v/v" was used as mobile phase at 0.5mL/min was found to be an appropriate mobile phase for separation of Camel Milk Dry powder. The column was maintained at ambient 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.

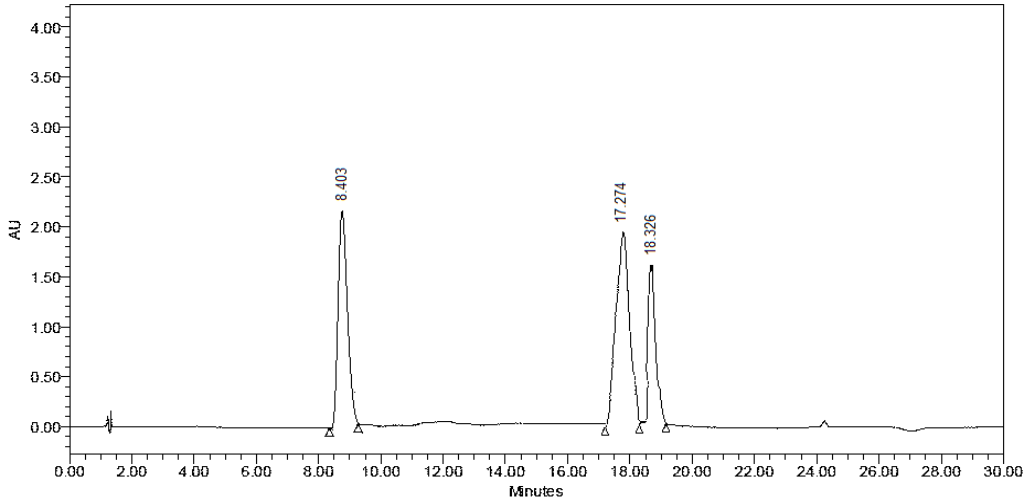
Preparation of Camel Milk Dry powder standard solution

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***

Camel Milk Dry powder in UPLC System

Camel Milk Dry powder	
System	UPLC
Stationary Phase	C18 column
"Mobile Phase"	"Chloroform and Methanol in the ratio of 30:70%v/v"
Diluents	Methanol
Injection volume	5µl
Temperature	Ambient
Flow rate	0.5 ml/min
UV detection	230nm
Retention Time	Lactoferrin – 17.274 mins; 18.236 mins Casein – 8.403 mins
Inference	"High column pressure were observed"



Chromatogram of standard preparation of Camel Milk Dry powder (Chloroform and Methanol in the ratio of 30:70%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	02.13	02.11	99.06	99.46%	0.27004	0.27%
100	04.13	04.09	99.51			
150	06.14	06.13	99.83			

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	17.385 ± 0.06; 18.364 ± 0.06
Theoretical plates ± % RSD	4833.37 ± 0.50; 6506.99 ± 0.50
Asymmetry ± % RSD	1.03 ± 0.05; 1.04 ± 0.05
Repeatability (% RSD)	0.46; 0.47

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."

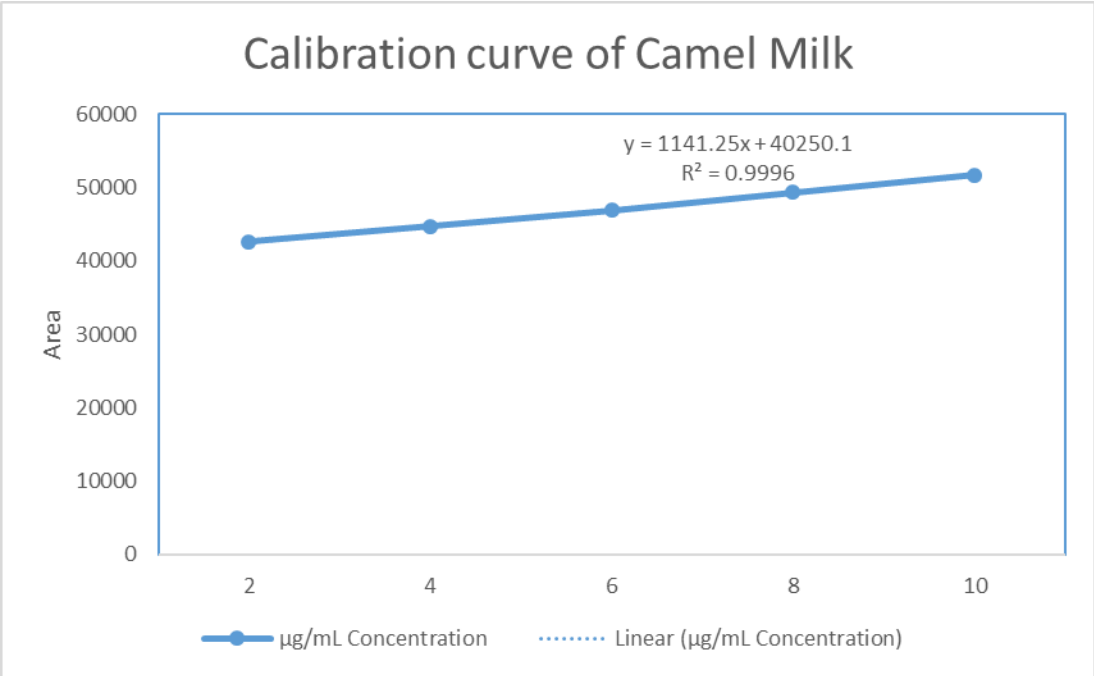
Method Precision

Replicate	*Camel Milk*		
S.No.	**Concentration Taken (µg/ml)**	**Area**	**%LC**
1	04.00	45748	99.99%
2		45731	99.96%
3		45767	99.93%
4		45679	99.86%
5		45692	99.81%
6		45752	99.76%
Average			99.88%
Std.Dev			0.090055
% RSD			0.09%
Standard weight			4mg
Standard potency			99.60%

Linearity

Camel Milk		
Linearity level	**Concentration in µg/mL**	**Area**
1	2 µg/mL	45767
2	4 µg/mL	50343
3	6 µg/mL	54920
4	8 µg/mL	59497
5	10 µg/mL	64073
Correlation co-efficient	0.9996	
Slope	1141.25	
Intercept	40250.1	

Linearity



Calibration Curve of Camel Milk

Robustness

Robustness Studies			
Parameter	**Value**	**Peak Area**	**% RSD**
Flow Rate	**Low**	45781	0.11%
	Actual	45767	
	Plus	45787	
Temperature	**Low**	45780	0.67%
	Actual	45773	
	Plus	45770	
Wavelength	**Low**	45769	0.07%
	Actual	45782	
	Plus	45789	

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	45796	0.46%	98.93%
	45801		99.10%
	45793		99.79%
Inter day precision	45850	0.47%	98.92%
	45816		99.09%
	45834		99.81%
Instrument:1 Acquity UPLC Waters,2695H	45823	0.42%	99.52%
	45789		99.69%
	45797		98.90%
Instrument:2 Agilent Technologies,1290	45836	0.41%	99.53%
	45791		99.67%
	45795		98.91%
Average			**99.23%**
Std.Dev			**0.3687**
%RSD			**0.37%**

Ruggedness

LOD and LOQ

LOD

LOD=3.3(SD of intercept/Slope)

Total numbers: 5

SE of Intercept: 1614.63

SD of Intercept: 724.04

LOD= 3.3*(724.04/ 1141.25)

LOD= 3.3*(0.06344)

LOD= 0.20936(µg/ml)

LOQ

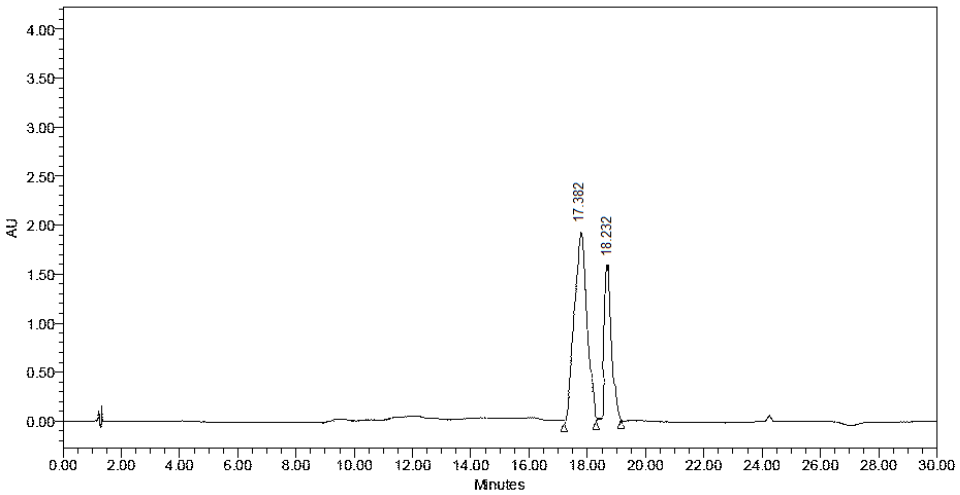
LOQ=10*(SD/S)

LOQ= 10*(724.04/ 1141.25)

LOQ= 0.6344(µg/ml)

ASSAY

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



ASSAY

$$\% \ Assay = \frac{26139}{28358} \times \frac{04.15}{100} \times \frac{1}{25} \times \frac{100}{04.25} \times \frac{25}{1} \times \textbf{Error!} \times 99.98 = 91.69\%$$

CONCLUSION

To be able to determine the quantitative amounts of process-related pollutants and Camel Milk corruption items in pharmaceutical formulations, a short specific, precise, precise, and sensitive approach was devised. After going through a stretch investigation, the debasement items of Camel Milk were successfully isolated from Camel Milk and its contaminants, and the mass equalizations were found to be satisfactory under all of the push conditions, demonstrating the method's ability to identify soundness under a variety of conditions.

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