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CHARACTERIZATION OF FORCE DEGRADATION ASSAY METHOD EVALUATION OF CAMEL MILK IN DRY POWDER DOSAGE FORM USING UPLC-MS/MS^n

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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. As long as you keep the flow rate constant, you'll see excellent resolution. Camel Milk Dry powder's PDA detector response was examined, and a wavelength of 247 nm was discovered to have the maximum sensitivity. For the separation of Camel Milk Dry powder, a 35:65 percent v/v solution combination of Ammonium formate and Methanol was employed as a mobile phase, moving at 0.8 mL/min. The temperature of the column was kept constant at room temperature.

KEYWORDS: Camel Milk Dry powder, Ammonium formate and Methanol.

INTRODUCTION

Chemical stability of Camel Milk dry powder is a matter of great concern as it affects the safety and efficacy of the finished drug product. Forced degradation studies provide data to support identification of possible degradants; degradation pathways and intrinsic stability of the Camel Milk dry powder molecule and validation of stability indicating analytical procedures. (ICH Q2 (R1), 2005).

A detailed literature revealed that several analytical methods have been reported for the determination of Camel Milk dry powder in pharmaceutical oral dosage forms. In our present knowledge, there is no method reported for the estimation forced degradation studies of Camel Milk dry powder in pharmaceutical oral dosage form by UPLC-MS/MS^n.

As per the stringent regulatory requirements recommended by the ICH and regulatory agencies, it is mandatory and important to identify and structurally characterize any impurity formed during production and stability testing, exceeding the identification threshold. Various analytical instruments and advanced hyphenated techniques are routinely used to carry out the impurity profile study.

The present work aims with the development of method to separate the degradation product by preparative UPLC and subjected to ESI-MS/MS. The study describes the separation of different impurities of Camel Milk dry powder, as well as the development and validation of a stability-indicating RP-UPLC method for the estimation of degradation and process-related impurities of Camel Milk dry powder. Forced degradation studies were performed on the drug product to show the stability-indicating nature of the method. These studies were performed in accordance with established ICH guidelines.

EXPERIMENTAL METHODOLOGY

Preparation of Standard Stocz 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 0.8 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 247 nm showing highest sensitivity.

The mixture of two solutions Ammonium formate and Methanol in the ratio of 35:65%v/v was used as mobile phase at 0.8mL/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

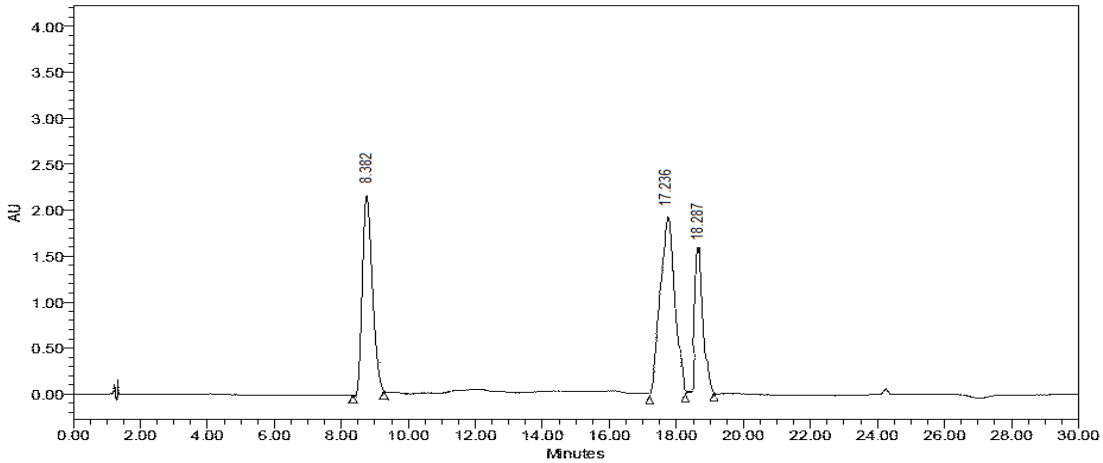
volumetric flask, add 50 ml of mobile phase and sonicate to dissolve it completely and then volume was made up to the mark with mobile phase to get 100 µg/ml of standard stock solution of working standard. Then it was ultrasonicated for 10 minutes and filtered through 0.20 µ membrane filter.

Preparation of Camel Milk Dry powder standard solution

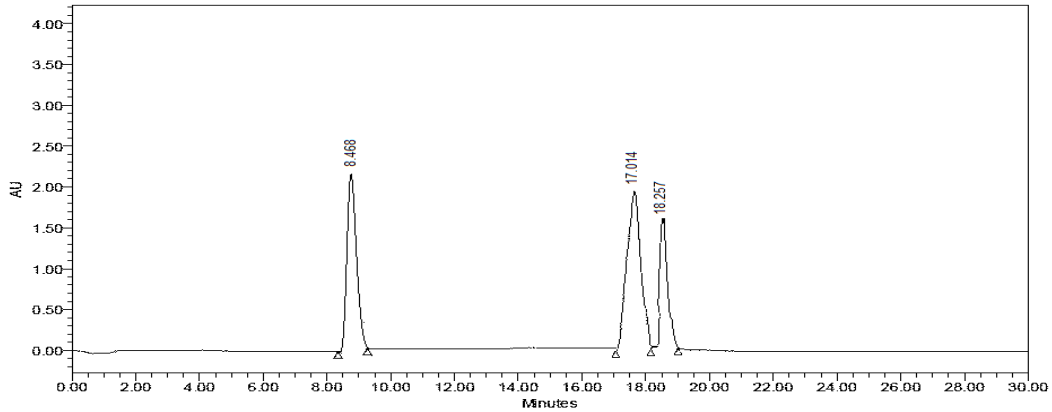
Weighed accurately about 10 mg of Camel Milk Dry powder and transfer to 100 ml volumetric flask, add 50 ml of mobile phase and sonicate to dissolve it completely and then volume was made up to the mark with mobile phase to get 100 µg/ml of standard stock solution of working standard. Then it was ultrasonicated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

Camel Milk Dry powder	
Method development by UPLC Optimised Trial	
System	UPLC
Stationary Phase	C18
"Mobile Phase"	"Ammonium formate and Methanol in the ratio of 35:65%v/v"
Injection volume	5µl
Temperature	Ambient
Flow rate	0.8mL/min
UV detection	247nm
Retention Time	Lactoferrin – 17.236 mins; 18.287 mins; Casein – 8.382 mins
Inference	"Better resolution of the peaks with clear base line separation was found."

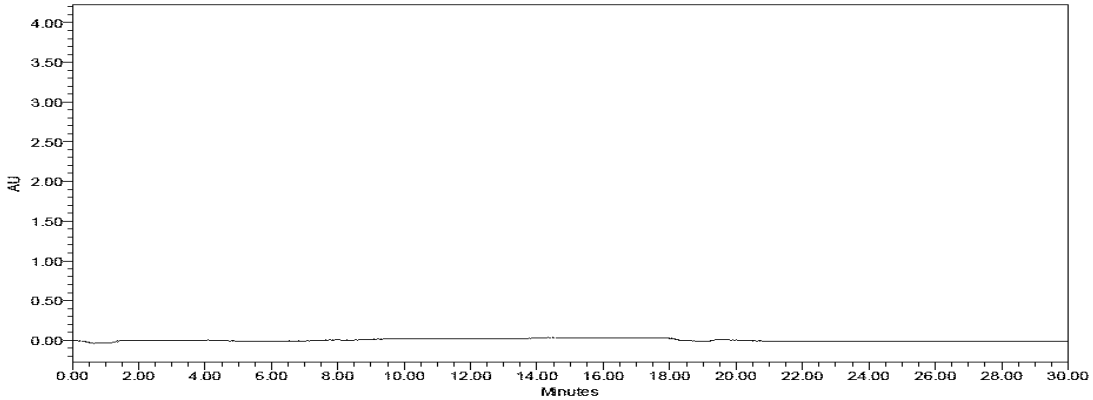
Method development of Camel Milk Dry powder in UPLC System



Chromatogram of standard preparation of Camel Milk Dry powder ("Ammonium formate and Methanol in the ratio of 35:65%v/v")



Chromatogram of test preparation of Camel Milk Dry powder (Ammonium formate and Methanol in the ratio of 35:65%v/v)

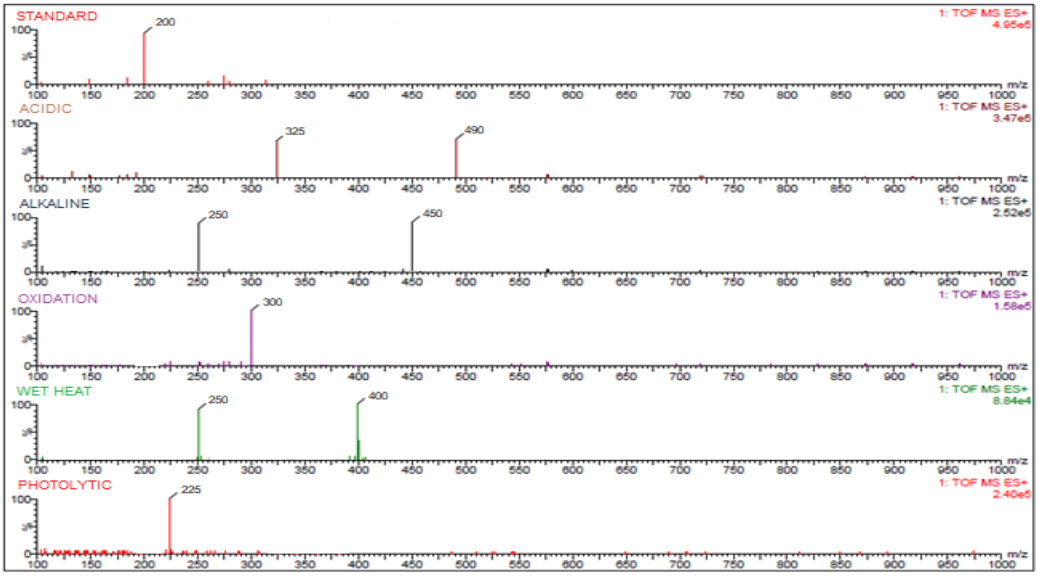


Blank Chromatogram of Camel Milk Dry powder
("Ammonium formate and Methanol in the ratio of 35:65%v/v")

Gradient Composition of Camel Milk Dry powder

Time Interval (Mins.)	Solvent – A% (Octane sulfonic acid buffer)	Solvent – B%(Mobile Phase)
0-5	0	100
5-15	25	75
15-25	15	85
25-30	0	100

Gradient Composition of Camel Milk Dry powder in UPLC System



LCMS

Analyte	Observed ion mass (Da)	Proposed formula	Calculated mass (Da)
Unknown	200.02	C8H9NO5	199.16
Unknown	225.74	C10H12NO5	226.21
Unknown	250.53	C11H8NO6	250.18
Unknown	300.42	C15H10NO6	300.24
Unknown	325.36	C17H11NO6	325.27
Unknown	405.45	C20H22NO8	404.39
Unknown	450.87	C22H28NO9	450.46
Unknown	490.72	C23H24NO11	490.44

LCMS Interpretation

SUMMARY OF RESULTS

Parameters	Acceptance Criteria	Value Found	Inference
Linearity Range (µg/ml)	Linear Regression	*Camel Milk* - 2-10 (µg/ml)	The concentration of linearity range was found to be Linear.
Correlation Coefficient	Correlation Coefficient of $r^2 \geq 0.995$	*Camel Milk* - 0.999	It is found to be under the acceptance criteria.
Method Precision (%RSD)	%RSD nmt 2%	*Camel Milk* - 0.02%	The Method Precision is found to be precised.
Accuracy (%Recovery)	The % recoveries should be in between 98-102% w/w	*Camel Milk* - 98.69%	The good recoveries shows the method is Accurate.
LOD (µg/ml)	Signal to noise ratio (S/N) ≥ 3.3	*Camel Milk* - 0.0029 µg/mL	It is found to be under the acceptance criteria.
LOQ (µg/ml)	Signal to noise ratio (S/N) ≥ 10.1	*Camel Milk* - 0.0091 µg/mL	It is found to be under the acceptance criteria.
Robustness (Low, Actual, High) (%RSD)	%RSD nmt 2%	*Camel Milk – (0.03%,* 0.02%, 0.02%)	With small deliberate variations in the method parameters has proven that the method is robust.
Intra Day (%RSD)	%RSD nmt 2%	*Camel Milk* - 0.02%	The repeatability is excellent, indicating the ruggedness of the method.
Inter Day (%RSD)	%RSD nmt 2%	*Camel Milk* - 0.01%	The reproducibility is excellent, indicating the ruggedness of the method.
Analysis of Formulation (% Assay)	The % Assay should be in between 98-102% w/w	*Camel Milk -91.69* %	Satisfactory quantitative detection of the analytes

SUMMARY OF RESULTS

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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