Application Note No. 081

The Automated Sample Preparation and Analysis of a Pharmaceutical Cream using the Focus Sample Processing Robot

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Introduction

The extraction of active ingredients from pharmaceutical products, especially creams, can often be a long and tedious process involving many steps and using strongly acidic and basic reagents.

An automated extraction method has been written closely following the customers existing manual method, the main difference being that the volumes have been reduced to allow the use of 10 mL headspace vials. The method uses the Focus Sample Processing Robot for the extraction of an active ingredient (AI) for the quality control of a pharmaceutical cream.

Instrumentation

- ATASGL Focus Robotic Sample Processor
- Agilent 6890 GC with split/splitless injector and FID
- Agilent ChemStation

The Focus is equipped with the dilutor-dispenser option, which enables the addition of larger volumes of reagents to the sample vial, than the 20 µL side-port syringe can hold. The Focus is software controlled and uses magnets to transport the vials between the sample tray and the agitator. Two vials are used per sample, the first contains the sample to which reagents are added to extract the active ingredients. The extracts are then transferred to a second vial and are back-extracted into a solvent that is suitable for injection into the GC. After all extractions are complete the syringe kit is changed and a low volume split injection is made.

Method

The sample preparation, using the Focus, is a multi-step method. The Focus is loaded with the four reagent vials (alkaline methanol, internal standard (IS), chloroform and acid); two wash solvents (methanol and chloroform); methanol to flush the dilutor; sample vials containing approximately 375 mg of cream; and an equivalent number of second sample vials which are empty.

The Focus adds alkaline methanol solution (2.5 mL) and IS (20 µL), transports the vial to the agitator and shakes the sample while it picks-up the chloroform (2.5 mL). After addition of the chloroform the active ingredient is extracted into the methanol and the layer is removed to a second vial. A second extraction of the chloroform is made with alkaline methanol (1.25 mL) and the two extracts are combined. The Focus transports the second vial, containing the extracts, to the agitator and shakes them before adding firstly acid (500 µL) then chloroform (2.5 mL). After all extractions have been performed and the syringe has been changed a 2 uL injection is made from the lower chloroform layer. The figure below outlines the process:

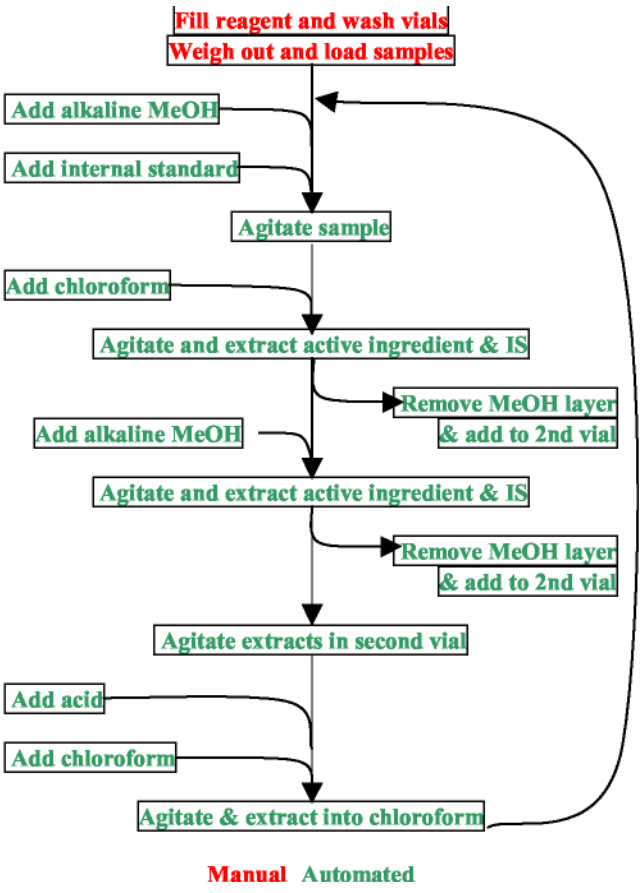

Figure: *Overview of the pharmaceutical cream extraction method*

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Performance Data

Table 1: System suitability calculations

	IS area	AI area	Ratio AI/IS
Cal 1	547.192	1406.862	2.5711
Cal 2	552.111	1413.317	2.5598
Cal 3	558.245	1427.342	2.5568
Cal 4	557.803	1421.862	2.5490
Cal 5	547.438	1410.582	2.5767
Cal 6	554.590	1425.088	2.5696
Average	552.8966	1417.5089	2.5638
Std Dev	4.8684	8.3903	0.0103
%RSD	**0.881**	**0.592**	**0.403**

Results

See Table 2 on page 3 for the results of 10 extracted samples and 1 extracted standard.

Conclusions

The preliminary results shown here indicate that the Focus system is suitable for the extraction of a pharmaceutical cream, giving %RSDs well far below the limit of 2% and assay results well within the limits of 0.9-1.1 mg/g. Further work will be carried out to determine the robustness of this technique.

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Results

Table 2: Results for 10 extracted samples and 1 extracted standard

ID	Sample weight (g)	IS peak area	AI peak area	Std area ratio AI/IS	Smp area ratio AI/IS	Assay result (mg/g)	Mean result (mg/g)	%LS
Cal		558.580	1426.096	2.5531				
Cal		556.927	1427.468	2.5631				
Sample 1	*0.39025*	580.707	1506.023		*2.5934*	*1.015*		*101*
Sample 1	*0.39025*	575.757	1494.195		*2.5952*	*1.015*	***1.02***	*102*
Cal		549.641	1416.939	2.5779				
Cal		552.887	1428.785	2.5842				
Sample 2	*0.40164*	578.057	1523.065		*2. 6348*	*1. 002*		*100*
Sample 2	*0.40164*	573.584	1521.406		*2. 6525*	*1. 008*	***1.01***	*101*
Cal		553.106	1431.543	2.5882				
Cal		553.108	1429.087	2.5837				
Sample 3	*0.38878*	563.515	1448.559		*2.5706*	*1. 010*		*101*
Sample 3	*0.38878*	567.769	1459.442		*2.5705*	*1. 010*	***1.01***	*101*
Cal		556.504	1431.586	2.5725				
Cal		534.145	1383.070	2.5893				
Sample 4	*0.37085*	573.403	1400.106		*2.4417*	*1. 005*		*101*
Sample 4	*0.37085*	572.828	1395.951		*2.4369*	*1.003*	***1.00***	*100*
Cal		560.123	1430.782	2.5544				
Cal		564.756	1447.565	2.5632				
Sample 5	*0.36315*	561.400	1340.135		*2.3871*	*1.004*		*100*
Sample 5	*0.36315*	576.219	1363.045		*2.3655*	*0.995*	***1.00***	*99*
Cal		569.292	1459.532	2.5638				
Cal		567.088	1454.506	2.5649				
Sample 6	*0.35901*	573.068	1348.141		*2.3525*	*1. 001*		*100*
Sample 6	*0.35901*	571.738	1347.061		*2.3561*	*1.002*	***1.00***	*100*
Cal		560.789	1436.828	2.5622				
Cal		555.699	1429.016	2.5716				
Sample 7	*0.38715*	591.867	1449.859		*2.4496*	*0.966*		*97*
Sample 7	*0.38715*	593.659	1453.460		*2.4483*	*0.966*	***0.97***	*97*
Cal		549.803	1417.701	2.5786				
Cal		550.739	1435.541	2.6066				
Sample 8	*0.38784*	564.631	1446.760		*2.5623*	*1. 009*		*101*
Sample 8	*0.38784*	572.087	1457.320		*2.5474*	*1. 003*	***1.01***	*100*
Cal		561.169	1444.675	2.5744				
Cal		552.708	1425.642	2.5794				
Sample 9	*0.37168*	600.103	1458.046		*2.4297*	*0.998*		*100*
Sample 9	*0.37168*	583.384	1418.301		*2.4312*	*0.999*	***1.00***	*100*
Cal		561.931	1442.681	2.5674				
Cal		565.071	1452.572	2.5706				
Sample 10	*0.39156*	584.051	1506.032		*2.5786*	*1.006*		*101*
Sample 10	*0.39156*	587.847	1515.846		*2.5786*	*1.006*	***1.01***	*101*
Cal		559.632	1442.107	2.5769				
Cal		602.012	1497.729	2.4879				

K Factor: **3.495**

% RSD (all calibrants): **0.8590**

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