# **Supporting Information**

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# An HPLC/CAD method for determination of fatty acids in metered dose inhalation products: a candidate leachable test method

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#### S1: The Interaction Between the Packaging System and The Dosage Form

Degree of concern associated with the	Likelihood of packaging component-dosage form interaction				
route of administration	High	Medium	Low		
Highest	Inhalation Aerosols and Sprays	Injections and Injectable Suspensions; Inhalation Solutions	Sterile Powders and Powders for Injection; Inhalation Powders		
High	High Transdermal Ointments and Patches	Ophthalmic Solutions and Suspensions; Nasal Aerosols and Sprays	-		
Low	Low Topical Solutions and Suspensions; Topical and Lingual Aerosols; Oral Solutions and Suspensions	-	Oral Tablets and Oral (Hard and Soft Gelatin) Capsules; Topical Powders; Oral Powders		

Table S1: The interaction between the packaging system and the dosage form

#### S2: Preparation of Mobile Phase

In order to determine the appropriate mobile phase to be used in the method, 1000.0 mL of purified water was taken for mobile phase A and 1000.0 mL of acetonitrile was taken for mobile phase B, and 0.1-1.0 mL (0.1 mL increments) of formic acid solution was added. As a result of the evaluation, 0.5 mL formic acid was found suitable for both phases. The applied gradient program is summarized in the Table 2.

Mobile Phase A: 0.5 mL of formic acid into the 1000.0 mL of pure water.

Mobile Phase B: 0.5 mL of formic acid into the 1000.0 mL of acetonitrile.

	· program	
Time (minute)	Mobile phase A (%)	Mobile phase B (%)
0.0	37.0	63.0
28.0	15.0	85.0
40.0	15.0	85.0
40.01	37.0	63.0
45.0	37.0	63.0

#### S3: Calculation

The following equations were used to calculate amount of sample.

$$\frac{Asmp}{Astd} \times \frac{Wstd}{100.0} \times \frac{2.5}{100.0} \times \frac{P}{100} \times \frac{10.0}{Wsmp} \times \frac{1}{L} \times 100 \times \text{CF} = mcg/canister$$

- Asmp : Impurity peak area obtained from the sample solution
- Astd : Stearic acid peak area obtained from standard solution
- Wstd : Standard weight (mg)
- P : Standard potens (%)
- L : Label value ( $\mu$ g/ canister)
- CF : Correction factor

#### **S4 : Validation Parameters**





Figure S1:. Blank chromatograph



Figure S2: Chromatograph of myristic acid



Figure S4: Chromatograph of palmitic acid



Figure S5: Chromatograph of oleic acid



Figure S6: Chromatograph of elaidic acid



Figure S7: Chromatograph of stearic acid



Figure S10: Chromatograph of unspike sample



Figure S11: Chromatograph of spike sample

# S4.2. AccuracyRresults

Level	Experimental Value	Theoretical Value	Recovery	Average	SD	RSD	
(%)	(µg/mL)	(µg/mL)	(%)	(%)	50	(%)	
	0.000331	0.000328	99.1				
LQ	0.000327	0.000331	101.2	99.8	1.2	1.2	
	0.000332	0.000329	99.1				
	0.007516	0.007536	100.3				
100	0.007430	0.007551	101.6	100.3	1.3	1.3	
	0.007556	0.007481	99.0				
	0.011274	0.011299	100.2				
150	0.011145	0.011569	103.8	101.1	2.4	2.4	
	0.011334	0.011251	99.3				
		Average	100.4				
		SD	1.6				
		% RSD	1.6				
		95% CI	1.2				

Table S3 : Accuracy results for myristic acid

Level	Experimental Value	Theoretical Value	Recovery	Average	SD	RSD
(%)	(µg/mL)	(µg/mL)	(%)	(%)	50	(%)
	0.000343	0.000352	102.6			
LQ	0.000343	0.000359	104.7	103.5	1.1	1.1
	0.000344	0.000355	103.2			
	0.007453	0.007515	100.8			
100	0.007454	0.007610	102.1	101.4	0.7	0.7
	0.007470	0.007574	101.4			
	0.011179	0.011054	98.9			
150	0.011179	0.011021	98.6	100.3	2.6	2.6
	0.011179	0.011544	103.3			
		Average	101.7			
		SD	2.0			
		% RSD	2.0			
		95% CI	1.5			

 Table S4 : Accuracy results for linoleic acid

Level	Experimental Value	Theoretical Value	oretical Value Recovery		SD	RSD
(%)	(µg/mL)	(µg/mL)	(%)	(%)	50	(%)
	0.000121	0.000120	99.2			
LQ	0.000121	0.000120	99.2	99.5	0.5	0.5
	0.000120	0.000120	100.0			
	0.007536	0.007596	100.8			
100	0.007536	0.007499	99.5	100.0	0.7	0.7
	0.007536	0.007515	99.7			
	0.011304	0.011256	99.6			
150	0.011304	0.011245	99.5	100.7	2.1	2.1
	0.011304	0.011652	103.1			
		Average	100.1			
		SD	1.2			
		% RSD	1.2			
		%95				
		0.9 95% CI				

Table S5 : Accuracy results for palmitic acid

Level	Experimental Value	Theoretical Value	Recovery	Average	SD	RSD
(%)	(µg/mL)	(µg/mL)	(%)	(%)	50	(%)
	0.000751	0.000781	104.0			
LQ	0.000753	0.000784	104.1	104.4	0.6	0.6
	0.000745	0.000783	105.1			
	0.007512	0.007572	100.8			
100	0.007525	0.007612	101.2	101.3	0.5	0.5
	0.007451	0.007588	101.8			
	0.011269	0.011263	99.9			
150	0.011269	0.011314	100.4	100.3	0.4	0.4
	0.011269	0.011333	100.6			
		Average	102.0			
		SD	1.9			
		% RSD	1.9			
		95% CI	1.5			

Table S6 : Accuracy results for oleic acid

Level	Experimental Value	Theoretical Value	Recovery	Average	SD	RSD
(%)	(µg/mL)	(µg/mL)	(%)	(%)	50	(%)
	0.000733	0.000741	101.1			
LQ	0.000731	0.000749	102.5	101.7	0.7	0.7
	0.000744	0.000755	101.5			
	0.007330	0.007415	101.2			
100	0.007330	0.007503	102.4	102.3	1.0	1.0
	0.007330	0.007562	103.2			
	0.010995	0.011263	102.4			
150	0.010995	0.011412	103.8	104.0	1.7	1.6
	0.010995	0.011632	105.8			
		Average	102.7			
		SD	1.5			
		% RSD	1.5			
		95% CI	1.2			

Table S7 : Accuracy results for elaidic acid

Level	Experimental Value	Theoretical Value	Recovery	Average	SD	RSD
(%)	(µg/mL)	(µg/mL)	(%)	(%)	50	(%)
	0.000735	0.000751	102.2			
LQ	0.000737	0.000766	103.9	103.1	0.9	0.9
	0.000737	0.000761	103.3			
	0.007354	0.007415	100.8			
100	0.007354	0.007462	101.5	101.5	0.7	0.7
	0.007354	0.007510	102.1			
	0.011031	0.011290	102.3			
150	0.011031	0.011312	102.5	102.5	0.2	0.2
	0.011031	0.011333	102.7			
		Average	102.4			
		SD	0.9			
		% RSD	0.9			
		95% CI	0.7			

 Table S8 : Accuracy results for arachidic acid

## S4.3. Repeatability Results

	C14:0	C18:2 <sup>Δ9, 12</sup>	C16:0	C18:1 n9	C18:1 n9t	C18:0	C20:0
1	81.917	75.868	79.464	76.809	75.402	79.371	76.962
2	75.734	75.340	81.171	76.427	74.273	78.454	79.416
3	75.457	73.254	75.447	71.807	70.349	75.690	72.608
4	75.933	71.712	74.810	71.756	70.463	74.319	73.547
5	80.113	76.023	80.483	76.858	74.994	79.705	75.065
6	80.226	75.812	80.829	77.022	75.391	79.092	74.580
Average	78.230	74.668	78.701	75.113	73.5	77.8	75.4
SD	2.840	1.776	2.833	2.588	2.415	2.225	2.473
% RSD	3.6	2.4	3.6	3.4	3.3	2.9	3.3
95% CI	2.981	1.864	2.974	2.716	2.535	2.335	2.596
Absolute Difference	9.3	4.5	2.1	4.4	3.8	2.8	3.1

**Table S9 :** Intermediate repeatability results

## S4.4. Linearity Results

				Average	Calculated	Difference	<b>Residual</b> of
Standard	Level	Ustanuaru	Astandard	Astandard	Area (V2)	Area	Squares
No		µg/IIIL		<b>(Y1)</b>	Alea (12)	(Y1-Y2)	$(Y1-Y2)^2$
			675633				
			681414				
1	100	0 0003304	648611	674616	1073368	30752	159003157504
1	LUQ	0.0005504	679544	074010	1075500	59152	139003137304
			665122				
			697369				
			5567639				
2	50%	0.0037610	5759085	5717088	5404766	312322	97545031684
			5824541				
			8445116				
3	80%	0.0060176	8842597	8583778	8253898	329880	108820814400
			8463622				
			10343010				
			10787787				
4	100%	0 0075220	10617506	10454527	10153310	301208	90726259264
-	10070	0.0073220	10261196	10434327	10103517	501200	50120235204
			10267666				
			10449998				
			11571383				
6	120%	0.0090264	11636340	11675309	12052741	377432	142454914624
			11818203				
			14900219				
			14935456				
7	150%	0.0112830	14989248	11721617	1/001070	167006	27064525076
1	15070	0.0112030	14729228	17/3707/	17/01077 107220 279043		2170 <del>1</del> 333010
			14330646				
			14523086				

## Table S10 : Linearity results for myristic acid



Figure S12: Linearity graphic for myristic acid

No         Level         µg/mL         Astandard         Astandard         Astandard         Area (Y2) (Y1)         Area         Squares           1         LOQ         0.0003560         1186184 1281414         1281414         108312         2195435         1087123         1181836417           1         LOQ         0.0003560         872203 1167083         1108312         2195435         1087123         1181836417           2         50%         0.0038612         11842762         11805848         11169489         636359         4049527768           3         80%         0.0061779         17839174         17821966         17100732         721234         5201784827	l of
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	es
1       LOQ       0.0003560       1186184 1281414         872203 1167083       1108312       2195435       1087123       1181836417         980198       1162788       1162788       1162788         11799368       11842762       11805848       11169489       636359       4049527768         2       50%       0.0038612       11842762       11805848       11169489       636359       4049527768         3       80%       0.0061779       17839174       17821966       17100732       721234       5201784822	$()^2$
1       LOQ       0.0003560 <sup>1281414</sup> <sup>872203</sup> <sup>1108312</sup> <sup>2195435</sup> <sup>1087123</sup> <sup>1181836417</sup> <sup>1181836417</sup> <sup>980198</sup> <sup>1162788</sup> <sup>11799368</sup> <sup>11799368</sup> <sup>117748560</sup> <sup>3</sup> <sup>80%</sup> <sup>0.0061779</sup> <sup>17839174</sup> <sup>17821966</sup> <sup>17100732</sup> <sup>721234</sup> <sup>520178482*</sup> <sup>11007116</sup> <sup>11007116</sup> <sup>111017116</sup> <sup>111017117</sup> <sup>111017117</sup> <sup>111017117</sup> <sup>111111717415</sup> <sup>1110171177415</sup> <sup>111017117415</sup> <sup>111017117415</sup> <sup>111017117415</sup> <sup>111017117415</sup> <sup>111017117415</sup> <sup>111017117415</sup> <sup>111017117415</sup> <sup>111017117415</sup> <sup>111017117415</sup> <sup>111017117415</sup> <sup>111017117415</sup> <sup>111017117415</sup> <sup>111017117415</sup> <sup>111017117415</sup> <sup>111017117415</sup> <sup>111017117415</sup> <sup>111017117415</sup> <sup>111017117415</sup> <sup>111017117415</sup> <sup>1110171174115</sup> <sup>1110171174115</sup> <sup>1110171174115</sup> <sup>1110171174115</sup> <sup>1110171174115</sup> <sup>1110171174115</sup> <sup>1110171174115</sup> <sup>1110171174115</sup> <sup>1110171174115</sup> <sup>1110171174115</sup> <sup>1110171174115</sup> <sup>1110171174115</sup> <sup>1110171174115</sup> <sup>1110171174115</sup> <sup>111111741174117411741174117411741174117</sup>	
1       LOQ       0.0003560	
1       1000       0.0003300       1167083       1168312       2193433       1087123       1181830417         980198       1162788       1162788       1162788       11799368       11799368         2       50%       0.0038612       11842762       11805848       11169489       636359       4049527768         11775415       117748560       117748560       17100732       721234       5201784822	17120
980198 1162788 11799368 2 50% 0.0038612 11842762 11805848 11169489 636359 4049527768 11775415 17748560 3 80% 0.0061779 17839174 17821966 17100732 721234 5201784822	. / 1 2 9
1162788         11799368         2       50%       0.0038612       11842762       11805848       11169489       636359       4049527768         11775415       117748560         3       80%       0.0061779       17839174       17821966       17100732       721234       5201784827	
11799368         2       50%       0.0038612       11842762       11805848       11169489       636359       4049527768         11775415       117748560         3       80%       0.0061779       17839174       17821966       17100732       721234       5201784827	
2       50%       0.0038612       11842762       11805848       11169489       636359       4049527768         11775415       117748560         3       80%       0.0061779       17839174       17821966       17100732       721234       5201784827	
11775415 17748560 <b>3</b> 80% 0.0061779 17839174 17821966 17100732 721234 5201784827	6881
17748560 <b>3</b> 80% 0.0061779 17839174 17821966 17100732 721234 520178482	
<b>3</b> 80% 0.0061779 17839174 17821966 17100732 721234 5201784827	
	2756
17878163	
22488110	
21832437	
<b>4</b> 100% 0.0077224 21878132 21998267 21054979 943288 8897922500	0011
22074044	00772230744
22015078	
21701802	
24801874	
<b>6</b> 120% 0.0092669 24883211 24901559 25009229 107667 115921828	2889
25019591	
29301794	
29933192	
<b>2</b> 9996724 <b>7</b> 150% 0.0115836 29834378 30910469 1106091 1223437300	)0281
29886760	/0201
29859459	
30028339	

Table S11 : Linearity results for linoleic acid



Figure S13: Linearity graphic for linoleic acid

Standard		Catandard	_	Average	Coloulated	Difference	<b>Residual of</b>
Stanuaru	Level	Ustanuaru	Astandard	Astandard		Area	Squares
INU		µg/IIIL		<b>(Y1)</b>	Area (12)	(Y1-Y2)	$(Y1-Y2)^2$
			1628545				
			1363729				
1	100	0.0001203	1548971	1542605	1/17581	125024	15631000576
1	LUQ	0.0001203	1493821	1342003	1417301	123024	13031000370
			1588301				
			1632265				
			26957404				
2	50%	0.0037550	26389675	26481217	26209179	272038	74004673444
			26096571				
			41086411				
3	80%	0.0060080	41616511	41472691	41576464	103773	10768835529
			41715152				
			52184335				
			52898343				
Λ	1000/	0.0075100	53427524	52049024	51921220	1227614	1507026122006
4	100%	0.0075100	53796877	53048934	51621520	1227014	1307030132990
			53674324				
			52312201				
			58193103				
6	120%	0.0090120	59376830	58737054	62066177	3329123	11083059949129
			58641229				
			78177132				
			79400563				
-	1500/	0.0112650	79895328	70241692	77 422 462	1000000	2260650569400
1	150%	0.0112650	79759205	/9241682	//433462	1808220	3209039368400
			79104755				
			79113109				

Table S12 : Linearity results for palmitic acid



Figure S14: Linearity graphic for palmitic acid

				Average	Calardatad	Difference	Residual of
Standard		Cstandard	Astandard	Astandard		Area	Squares
No	Level	µg/mL		<b>(Y1)</b>	Area (¥2)	(Y1-Y2)	$(Y1-Y2)^2$
			1133048				
			1363729				
1	1.00	0.0007840	1440269	1222220	2214405	801265	704353300225
1	LUQ	0.0007840	1319854	1525250	2214495	495 891265 794353300225	794555500225
			1408024				
			1274458				
			25006845				
2	50%	0.0039700	24838947	24631082	24866015	234933	55193514489
			24047454				
			42227126				
3	80%	0.0063520	42008635	42387945	41801332	586613	344114811769
			42928075				
			55935785				
			53091501				
4	100%	0 0079400	56386072	54928125	53091544	1836581	3373029769561
-	10070	0.0077400	53616290	34920123	550715++	1050501	3373029709301
			56351012				
			54188089				
			64475515				
6	120%	0.0095280	64858922	64898111	64381756	516355	266622486025
			65359895				
			79953299				
			79715396				
7	150%	0.0119100	78431241	79503722	81317073	1813351	3288241849201
,	15070	0.0117100	81072685	17505122	01517075	1013331	52002+10+7201
			78466433				
			79383279				

 Table S13 : Linearity results for oleic acid



Figure S15: Linearity graphic for oleic acid

Standard No	Level	Cstandard µg/mL	Astandard	Average Astandard (Y1)	Calculated Area (Y2)	Difference Area (Y1-Y2)	Residual of Squares (Y1-Y2) <sup>2</sup>
1	LOQ	0.0007494	1044437 1168025 1062827 922207 1169963 1197526	1094164	3850073	2755909	7595034416281
2	50%	0.0035644	28072977 28524726 27196923	27931542	25950552	1980990	3924321380100
3	80%	0.0057030	44492581 45355580 44776577	44874913	42740635	2134278	4555142581284
4	100%	0.0071288	55533229 55936389 55410339 55875528 56304105 55559594	55769864	53934547	1835317	3368388490489
6	120%	0.0085546	63120392 63276102 63503776 78009028	63300090	65128459	1828369	3342933200161
7	150%	0.0106932	79532049 81000564 80510277 81392095 82869394	80552235	81918542	1366307	1866794818249

Table S14 : Linearity results for elaidic acid



Figure S16: Linearity graphic for elaidic acid

Standard		Cetandard	Average Differen		Difference	<b>Residual</b> of				
No	Lovol	Ustanuaru	Astandard	Astandard	A rea (V2)	Area	Squares			
INU	Level	µg/mL		(Y1)	Alea (12)	(Y1-Y2)	Residual of         Squares         (Y1-Y2)²         20015029601856         9709312664529         5843489162561			
			1029871							
			1051463							
1 I	100	0 0008259	1038164	1030052	5503868	1173816	20015029601856			
	LUQ	0.0000237	916825	1050052	5505000	475010	20013027001030			
			1115018							
			1028973							
			39485598							
2	50%	0.0037410	39108731	39069658	35953681	3115977	9709312664529			
			38614644							
			61949769							
3	80%	0.0059856	61770633	61817086	59399755	2417331	5843489162561			
			61730857							
			78702228							
			78632326							
4	100%	0.0074820	78152813	78591612	75030471	3561141	12681725221881			
-	10070		78816638							
			78572844							
			78672821							
			89581471							
6	120%	0.0089784	89173427	89421861	90661187	1236326	1535928934276			
			89510686							
			110126356							
			109570086							
7	150%	0.0112230	110393434	110725954	114107261	3381307	11433237028249			
,	15070	0.0112230	110480788	110120704	117107201	5501507	20015029601856 9709312664529 5843489162561 12681725221881 1535928934276 11433237028249			
			111545288							
			112239772							

Table S15 : Linearity results for stearic acid



Figure S17: Linearity graphic for stearic acid

		Catandard		Average		Difference	<b>Residual of</b>
Standard	Louol		Astandard	Astandard	Calculated	Area	Squares
No	Level	µg/mL		(Y1)	Area (Y2)	(Y1-Y2) (Y1	(Y1-Y2) <sup>2</sup>
			995044				
			1131110				
1	100	0.0007402	1251520	1121001	2799061	1667970	2781700226000
1	LUQ	0.0007492	1051459	1121091	2/88901	100/8/0	2781790550900
			1135631				
			1161781				
			37613617				
2	50%	0.0036927	37637812	37533816	34465564	3068252	9414170335504
			37350018				
			58083826				
3	80%	0.0059082	57310204	57617747	57537265	80483	6477513289
			57459210				
			68892914				
			68160596				
1	100%	0.0073853	68610434	68340405	72010440	4570035	20885210001225
-	10070	0.0073833	68288436	08349405	72919440	4370033	20865219901225
			68125078				
			68018969				
			89983567				
6	120%	0.0088624	89860895	90281003	88301615	1979388	3917976854544
			90998548				
			111418944				
			111379438				
7	150%	0.0110780	112289960	1118/1151	11137/1357	166791	217896638//36
	15070	0.0110780	111043858	111041151	111374337	400794	217890038430
			112072036				
			112842672				

Table S16	:	Linearity	results	for	arachidic	acid
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Figure S18: Linearity graphic for arachidic acid