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Development and Validation of New Analytical Method for the Determination of Particle Size Distribution of Metformin Hydrochloride Using Laser Based Particle Size Analyzer

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Authors' contributions

This work was carried out in collaboration between all authors. Author AG designed the study and literature search along with author SJ. Authors MG and AL wrote the protocol. Authors SF and SP carried out the experiment. All author involved in statistical analysis, wrote the protocol and wrote the first draft of manuscript and subsequent revision. All the authors read and approved the final manuscript.

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Short Communication

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ABSTRACT

Metformin Hydrochloride has characteristic nature of lump formation and determination of particle size with reproducible result is difficult and particle size determination method is not reported in literature. Hence novel rugged and reproducible method has been developed for the determination of particle size distribution of Metformin Hydrochloride.The wet method using Isopar G as dispersant has been developed and validated as per International conference on Harmonization guidelines (Q2R1) and found out robust and reproducible with % RSD of d(10), d(50) and d(90)

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values found within acceptance limit ranges from 6.05% to 21.84% for d(0.1), 3.04% to 9.87% for d(0.5) and 4.69% to 14.32% for d(0.9) in validation.
The described method is accurate and validated and successfully applied for the determination of particle size distribution of Metformin Hydrochloride.

Keywords: Metformin hydrochloride; method development; method validation; particle size analyzer (PSA).

1. INTRODUCTION

Metformin, marked under the trade name Glucophage among others, is the first line medication for the treatment of type-2 diabetes, particularly in overweight people. It is also used in the treatment of polycystic ovary syndrome. Limited evidence suggests Metformin may prevent the cardiovascular disease and cancer complications of diabetes, [1,2].

Relative bioavailability [3], 3extended-released along with bioequivalence [4] and immediate released is related to particle size of Metformin hydrochloride [5]. Literature survey done for Particle size distribution method for Metformin Hydrochloride and found that particle size determination method is not available. Therefore, study was carried out to develop a method to determine particle size distribution of Metformin Hydrochloride by Particle size analyzer [6,7] and further validation of the method, [8] was carried out.

2. OBJECTIVES OF THE STUDY

The objective of the study was to developed method to determine the particle size distribution of Metformin Hydrochloride by particle size analyzer. Also to demonstrate that the procedure when correctly applied produce results that are fit for purpose by performing validation of the method.

3. MATERIALS AND REAGENT

Metformin Hydrochloride, soybean Lecithin, IsoparG was obtained from Indoco Remedies Ltd, Navi Mumbai, India.

4. ANALYTICAL METHOD DEVELOPMENT

The primary goal was to develop method to obtain the most stable, reproducible, consistent method. Various dispersant tried for the Metformin Hydrochloride on the basis of solubility [9,10]. Isopar G is found well suitable dispersant to develop method for determination of particle size distribution of Metformin Hydrochloride. Various trial were taken during method development as mention below:

Table 1. Material and Reagent details

Sr. No	Name	Manufacture name	Grade
1	Metformin Hydrochloride	Indoco Remedies Limited, Navi-Mumbai India	Inhouse
2	Soyabean Lecithin	ACROS Organics	GR
3	IsoparG	Sai Traders	GR

4.1 Trial No -1

4.1.1 Instrument parameter

Analysis is performed as per below described method for Dry dispersion unit. RI is kept as per Chem spider.

Table-2. Instrument/method parameter details

Method Parameters	
Equipment	Malvern Mastersizer
Model	Mastersizer 2000
Sample handling unit	Dry Dispersion Unit
Sample model	Scirocco 2000
Sample refractive index	1.576
Sample absorption	0.1
Dispersant Refractive Index	1.000
Sample measurement time	5 seconds
Measurement Snaps	5,000
Background Measurement Time	5 seconds
Background Snaps	5,000
Vibration Feed Rate	40%
Air Pressure	2.0
Obscuration range	1-6%

4.1.2 Procedure

Transferred 1 to 2 g of sample into the sample tray with the help of a cleaned spatula and analysis repeated twice to check difference in d(10), d(50), and d(90) values and carried out measurements.

4.1.3 Observation

The weighted residual is less than 1% and also obscuration value was well within limit. Sample gets stuck to sample tray due to static charge, sample flow was not uniform and produces air bubble. Hence, the results were not reproducible. Next trial was performed by wet method.

Table-3. Observation table of Trial No-1

Measurement no	d(0.1)µ	d(0.5)µ	d(0.9)µ
1	7.823	39.156	844.141
2	5.745	30.914	213.877
3	5.029	32.379	931.963

4.2 Trial No-2

Dry Technique was not suitable, hence next trial was made with wet technique. All parameters were kept as per Trial-1 for wet dispersion and Isopropyl Alcohol and Tween 20 were used as dispersant and surfactant respectively. RI is kept as per chem. spider reference.

Table 4. Instrument/Method parameter details

Method Parameters	
Equipment	Malvern Mastersizer
Model	Mastersizer 2000
Sample handling unit	Wet Dispersion Unit
Sample model	Hydro 2000S
Sample refractive index	1.576
Sample absorption	0.1
Dispersant name	Isopropyl Alcohol
Dispersant Refractive Index	1.390
Sample measurement time	10 seconds
Measurement Snaps	10,000
Background Measurement Time	10 seconds
Background Snaps	10,000
Obscuration range	10-30%
No. of measurement cycles	3
Create average Result	Tick on this Box
Stirrer speed	2000 rpm

4.2.1 Procedure

Weighed about 50 mg of sample and transferred in 50 ml beaker added 2-3 drop of Tween-20. Prepared a paste and added 10 ml dispersant. Stirred well and sonicated externally for 1 minutes to prepared homogeneous solution. Sample was added in the dispersion unit when "Add sample under Obscuration" message was shown by the instrument. Results obtained are derived in Table No. 5

4.2.2 Observation

The weighted residual were less than 1%, but obscuration value decreases gradually due to particles dissolved in dispersant. Hence, next trial was carried by changing the dispersant and surfactant.

Table 5. Observation table of Trial No-1

Measurement no	d(0.1)µ	d(0.5)µ	d(0.9)µ
1	14.703	41.176	93.307

4.3 Trial No-3

All parameter were kept as per Trial No. – 2, only changed the dispersant to Isopar G and Lecithin solution as surfactant.

4.3.1 Preparation of lecithin solution

4 gm of Lecithin granules dissolved in 100 ml of Isopar G.

4.3.2 Procedure

Weighed 50 mg of sample and transferred in 50 ml beaker added 2-3 drops dispersant and then added 10 ml of Lecithin solution. Sonicated externally for 1 minute to form homogeneous solution. Sample solution was added in the dispersion unit when "Add sample under Obscuration" message was shown by the instrument software and performed the analysis. Obtained results are reported in Table No.–6. Analysis was repeated to check difference in d(10), d(50), and d(90) values and carried out measurements.

4.3.3 Observation

The obtained results of d(10), d(50), and d(90) values of analysis in twice are close to each other, weighted residue is less than 1% and obscuration is found satisfactory. To check the

reproducibility of method six replicate of sample was analysed and results were found reproducible. Hence, further method suitability can be confirmed by performing validation.

Table 6. Observation table of Trial No-3

Measurement no.	d(0.1)µ	d(0.5)µ	d(0.9)µ
1	2.653	20.413	55.270
2	2.778	20.654	56.543

5. METHODS VALIDATION

The validation work was conducted according to the ICH (International Conference on Harmonization) guidelines Q2R1. The method validation parameters include Precision, Intermediate Precision, Robustness and Batch Analysis.

5.1 Method Validation Parameters

5.1.1 Method Precision
5.1.2 Intermediate Precision
5.1.3 Robustness
5.1.4 Batch Analysis

5.2 Method Precision

5.2.1 Procedure

Determined the particle size of six precision samples as per above method and recorded the particle size for d(0.1), d(0.5) and d(0.9) in Table – 7

5.2.2 Results

The % RSD of d(0.1) particle size values is found not be more than 30, for d(0.9) particle size values is found not more than 15 and d(0.5) particle size values is found not more than 10.Results of Method Precision parameter tabulated in Table-7

5.2.3 Acceptance criteria

The % RSD of d(0.1) particle size values should not be more than 30, for d(0.9) particle size values should not be more than 15 and d(0.5) particle size values should not be more than 10.

5.3 Intermediate Precision

5.3.1 Procedure

Determined the particle size of six Intermediate precision samples as per above method and recorded the particle size for d(0.1), d(0.5) & d(0.9) particles in Table-8.

5.3.2 Results

The % RSD of d(0.1) particle size values is found not be more than 30, for d(0.9) particle size values is found not more than 15 and d(0.5) particle size values is found not more than 10.Results of Method Precision parameter tabulated in Table 8.

5.3.3 Acceptance criteria

The % RSD of d(0.1) particle size values should not be more than 30, for d(0.9) particle size values should not be more than 15 and d(0.5) particle size values should not be more than 10.

5.4 Robustness

5.4.1 Robustness-1 (change in stirrer speed to 1800 rpm)

5.4.1.1 Procedure

Determined the particle size of three replicates as per method of analysis, only change the stirrer speed to 1800 rpm and recorded particle Size for d(0.1), d(0.5) & d(0.9) in Table 9.

5.4.1.2 Results

The % RSD of d(0.1) particle size values is found not more than 30, for d(0.9) particle size values is found not more than 15 and d(0.5) particle size values is found not more than 10.Results of Robustness-1 parameter tabulated in Table 9.

5.4.1.3 Acceptance criteria

The % RSD of d(0.1) particle size values should not be more than 30, for d(0.9) particle size values should not be more than 15 and d(0.5) particle size values should not be more than 10.

5.4.2 Robustness-2 (Change in stirrer speed to 2200 rpm)

5.4.2.1 Procedure

Determined the particle size of three replicates as per method of analysis, only change the stirrer speed to 2200 rpm and recorded the particle Size for d(0.1), d(0.5) & d(0.9) in Table 10.

Table 7. Results of method precision

Sample ID	Particle size (µm)		
	d(0.1)	d(0.5)	d(0.9)
Precision-1	3.330	26.378	75.539
Precision-2	3.375	24.738	66.956
Precision-3	3.934	28.748	83.402
Precision-4	3.323	23.451	58.767
Precision-5	2.886	23.078	59.699
Precision-6	3.094	24.008	63.120
Average	3.324	25.067	67.914
% RSD	10.59	8.57	14.32

Table 8. Results of intermediate precision

Sample ID	Particle size (µm)		
	d(0.1)	d(0.5)	d(0.9)
Intermediate precision-1	3.797	27.484	76.727
Intermediate precision-2	3.585	27.519	83.277
Intermediate precision-3	3.331	26.740	83.708
Intermediate precision-4	3.218	25.974	74.379
Intermediate precision-5	3.449	25.921	72.335
Intermediate precision-6	3.342	25.719	79.284
Average	3.454	25.560	78.285
%RSD	6.05	3.04	5.95
Precision-1	3.330	26.378	75.539
Precision-2	3.375	24.738	66.956
Precision-3	3.934	28.748	83.402
Precision-4	3.323	23.451	58.767
Precision-5	2.886	23.078	59.699
Precision-6	3.094	24.008	63.120
Cumulative Average	3.389	25.813	73.099
Cumulative % RSD	8.39	6.71	12.40

Table 9. Results of robustness-1

Sample ID	Particle size (µm)		
	d(0.1)	d(0.5)	d(0.9)
Robustness-1(1)	3.463	24.037	71.839
Robustness-1(2)	2.616	21.519	57.086
Robustness-1(3)	2.814	22.273	58.891
Average	2.964	22.610	62.605
% RSD	14.95	5.72	12.85
Precision-1	3.330	26.378	75.539
Precision-2	3.375	24.738	66.956
Precision-3	3.934	28.748	83.402
Precision-4	3.323	23.451	58.767
Precision-5	2.886	23.078	59.699
Precision-6	3.094	24.008	63.120
Cumulative Average	3.204	24.248	66.144
Cumulative % RSD	12.44	9.04	13.72

5.4.2.2 Results

The % RSD of d(0.1) particle size values is found not more than 30, for d(0.9) particle size values is found not more than 15 and d(0.5) particle size values is found not more than 10. Results of Robustness-2 parameter tabulated in Table 10.

Table 10. Results of Robustness-2

Sample ID	Particle size (µm)		
	d(0.1)	d(0.5)	d(0.9)
Robustness-2(1)	3.587	24.682	63.607
Robustness-2(2)	4.025	27.644	74.345
Robustness-2(3)	3.664	28.068	82.826
Average	3.759	26.798	73.593
% RSD	6.22	6.88	13.09
Precision-1	3.330	26.378	75.539
Precision-2	3.375	24.738	66.956
Precision -3	3.934	28.748	83.402
Precision -4	3.323	23.451	58.767
Precision -5	2.886	23.078	59.699
Precision -6	3.094	24.008	63.120
Cumulative Average	3.469	25.644	69.807
Cumulative % RSD	10.73	8.26	13.62

Table 11. Results of Robustness-3

Sample ID	Particle size (µm)		
	d(0.1)	d(0.5)	d(0.9)
Robustness-3(1)	4.261	26.551	67.353
Robustness-3(2)	3.882	25.187	61.315
Robustness-3(3)	3.647	24.888	64.544
Average	3.930	25.542	64.404
% RSD	7.88	3.47	4.69
Precision -1	3.330	26.378	75.539
Precision -2	3.375	24.738	66.956
Precision -3	3.934	28.748	83.402
Precision -4	3.323	23.451	58.767
Precision -5	2.886	23.078	59.699
Precision -6	3.094	24.008	63.120
Cumulative Average	3.526	25.225	66.744
Cumulative % RSD	12.47	7.02	12.03

5.4.2.3 Acceptance criteria

The % RSD of d(0.1) particle size values should not be more than 30, for d(0.9) particle size values should not be more than 15 and d(0.5) particle size values should not be more than 10.

5.4.3 Robustness-3 (change in obscuration range to 10-15%)

5.4.3.1 Procedure

Determined the particle size of three replicates as per method of analysis, only change the obscuration range to 10 – 15%. and recorded the particle size for d(0.1), d(0.5) & d(0.9) in Table 11.

5.4.3.2 Results

The % RSD of d(0.1) particle size values is found not more than 30, for d(0.9) particle size values is found not more than 15 and d(0.5) particle size values is found not more than 10.Results of Robustness-3 parameter tabulated in Table-11

5.4.3.3 Acceptance criteria

The % RSD of d(0.1) particle size values should not be more than 30, for d(0.9) particle size values should not be more than 15 and d(0.5) particle size values should not be more than 10.

5.4.4 Robustness-4 (change in obscuration range to 25-30%)

5.4.4.1 Procedure

Determined the particle size of three replicates as per method of analysis, only changed the obscuration range to 25 – 30 % and recorded the particle size for d(0.1), d(0.5) & d(0.9) in Table 12.

5.4.4.2 Results

The % RSD of d(0.1) particle size values is found not more than 30, for d(0.9) particle size values is found not more than 15 and d(0.5) particle size values is found not more than 10.Results of Robustness-4 parameter tabulated in Table-12.

5.4.4.3 Acceptance criteria

The % RSD of d(0.1) particle size values should not be more than 30, for d(0.9) particle size values should not be more than 15 and d(0.5) particle size values should not be more than 10.

5.4.5 Robustness - 5 (change in sample measurement time to 9 seconds from 10 seconds)

5.4.5.1 Procedure

Determined the particle size of three replicates as per method of analysis, only changed the sample measurement time to 3 seconds and recorded the particle size for d(0.1), d(0.5) & d(0.9) in Table-13.

5.4.5.2 Results

The % RSD of d(0.1) particle size values is found not more than 30, for d(0.9) particle size values is found not more than 15 and d(0.5) particle size values is found not more than 10.Results of Robustness-5 parameter tabulated in Table 13.

5.4.5.3 Acceptance criteria

The % RSD of d(0.1) particle size values should not be more than 30, for d(0.9) particle size values should not be more than 15 and d(0.5) particle size values should not be more than 10.

Table 12. Results of Robustness-4

Sample ID	Particle size (µm)		
	d(0.1)	d(0.5)	d(0.9)
Robustness-4(1)	4.596	27.772	70.73
Robustness-4(2)	5.770	29.673	75.482
Robustness-4(3)	5.467	28.466	77.663
Average	5.278	29.304	74.625
% RSD	11.55	3.36	4.75
Precision -1	3.330	26.378	75.539
Precision -2	3.375	24.738	66.956
Precision -3	3.934	28.748	83.402
Precision -4	3.323	23.451	58.767
Precision -5	2.886	23.078	59.699
Precision -6	3.094	24.008	63.12
Cumulative Average	3.975	26.479	70.151
Cumulative % RSD	26.68	9.56	12.22

Table-13. Results of Robustness-5

Sample ID	Particle size (µm)		
	d(0.1)	d(0.5)	d(0.9)
Robustness-5(1)	4.663	27.208	66.641
Robustness-5(2)	4.629	27.966	67.68
Robustness-5(3)	5.287	30.527	76.289
Average	4.860	28.567	70.203
% RSD	7.62	6.09	7.54
Precision-1	3.330	26.378	75.539
Precision-2	3.375	24.738	66.956
Precision-3	3.934	28.748	83.402
Precision-4	3.323	23.451	58.767
Precision-5	2.886	23.078	59.699
Precision-6	3.094	24.008	63.12
Cumulative Average	3.836	26.234	68.677
Cumulative % RSD	21.84	9.87	11.96

5.4.6 Robustness - 6 (change in sample measurement time to 11 seconds from 10 seconds)

5.4.6.1 Procedure

Determined the particle size of three replicates as per method of analysis, only changed the sample measurement time to 7 seconds and recorded the particle size for d(0.1), d(0.5) & d(0.9) in Table 14.

5.4.6.2 Results

The % RSD of d(0.1) particle size values is found not more than 30, for d(0.9) particle size values is found not more than 15 and d(0.5) particle size values is found not more than 10.Results of Robustness-6 parameter tabulated in Table 14.

5.4.6.3 Acceptance criteria

The % RSD of d(0.1) particle size values should not be more than 30, for d(0.9) particle size values should not be more than 15 and d(0.5) particle size values should not be more than 10.

6. RESULTS AND DISCUSSION

As method for determination of particle size distribution of Metformin Hydrochloride was developed and validated. During development initial Trial-1 was taken by using dry dispersion technique results obtained were found inconsistent. Therefore, the next trial was conducted as Trial-2 by using wet dispersion technique results obtained were found within acceptance criteria but obscuration value decreases gradually due to particles dissolved in dispersant. Hence, next trial was carried as Trial-3 by changing the dispersant to Isopar G and Lecithin solution as surfactant, results obtained were found within acceptance criteria. Thus, Trial-3 was frozen for determination of particle size distribution of Metformin Hydrochloride and further method suitability were confirmed by method validation performing parameters such as Precision, Intermediate Precision and Robustness.

Table-14. Results of Robustness-6

Sample no.	Particle size (µm)		
	d(0.1)	d(0.5)	d(0.9)
Robustness-6(1)	4.037	27.305	70.917
Robustness-6(2)	4.454	28.689	71.961
Robustness-6(3)	4.999	28.558	75.863
Average	4.497	28.184	72.914
% RSD	10.73	2.71	3.58
Precision -1	3.330	26.378	75.539
Precision -2	3.375	24.738	66.956
Precision -3	3.934	28.748	83.402
Precision -4	3.323	23.451	58.767
Precision -5	2.886	23.078	59.699
Precision -6	3.094	24.008	63.12
Robustness-6(1)	4.037	27.305	70.917
Robustness-6(2)	4.454	28.689	71.961
Robustness-6(3)	4.999	28.558	75.863
Cumulative Average	3.715	26.106	69.580
Cumulative % RSD	18.64	8.95	11.77

In method validation, method was found précised with the results obtained were % RSD 10.59% for d(0.1), 8.57% for d(0.5) and 14.32% for d(0.9).In intermediate precision % RSD obtained were 6.05% for d(0.1), 3.04% for d(0.5) and 5.95% for d(0.9). Also, Cumulative % RSD obtained were 8.39% for d(0.1), 6.71% for d(0.5) and 5.12.40% for d(0.9). Also the method found Robust and the results obtained were % RSD ranges from 6.22% to 21.84% for d(0.1), 3.47% to 9.87for d(0.5) and 4-.69% to 12.85% for d(0.9).

7. CONCLUSION

The analytical method validation for Particle size of Metformin Hydrochloride by Particle size analyzer (Wet dispersion) was carried out by performing the parameters Precision, Intermediate Precision and Robustness. All the

data has been compiled and found to be satisfactory. Hence, method developed for the particle size method can be suitably used for analysis of Metformin Hydrochloride active pharmaceutical ingredient.

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CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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