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Short communication

Discriminatory power of an in-house developed method for *in vitro* release test (IVRT) used for analyzing topical product

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Introduction

In-house developed method for *in vitro* release test (IVRT) takes essential part of the process of development of topical products for local use. Using appropriate receptor media and inert membrane, this test provides information about medicine release rate and cumulative amount of active substance released from infinite dose of sample applied on the membrane in last sampling point of the test. [1]Drug release rate is the slope obtained from the cumulative amount of active substance released versus the square root of time for the linear portion of the drug release profile. (European Medicine Agency, Draft guideline on quality and equivalence of topical products, 2018). Test product and reference product are similar when they have similar release rate and cumulative amount of active substance.

According to EMA's Draft guideline on quality and equivalence of topical products, validation of method should include analysis of discriminatory power of the method. Discriminatory power of method should be demonstrated by analyzing formulations with changes in quality attributes, critical manufacturing variables or quantitative excipient composition. (European Medicine Agency, Draft guideline on quality and equivalence of topical products, 2018).

The aim of this study was to confirm the discriminatory power of the in-house developed IVRT method. The IVRT method has to be able to discriminate active substance release rates from non-similar formulations. IVRT sensitivity is the ability to detect changes in the release rate, as a function from concentration of active substance in the formulation. IVRT method is sensitive if it consistently identifies higher or lower rates of release for test formulations with increased or decreased concentrations of active substance and test formulations with different viscosity, relative to the proposed formulation.

If the method is discriminative, it can be valuable tool for development of topical formulation, quality control and comparison of test product and reference product.

Materials and methods

Materials

IVRT is performed on four laboratory trials. First trial is named test product and it is used for comparison with test formulations that have changes in viscosity and concentration of active substance. Test product has concentration of active substance same as referent product and similar viscosity. Second trial has 50% less active substance then test product. Third laboratory trial has 100% more active substance then test product. Last laboratory trial has different viscosity then test product.

IVRT

IVRT was performed on vertical diffusion cell (Franz cell). Test was performed on temperature that complies with the temperature of human body skin which is 32±0.5°C. Media with proved sink conditions that was used for analysis is 10% phosphate buffered saline (PBS). Speed of magnetic stirrer was 400 rpm. Membrane that acts inert with active substance was Nuclepore Track-Etch Membrane. During test, samples are taken in 10 different sampling time points: 10 min, 20 min, 30 min,

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60 min, 90 min, 120 min, 150 min, 180 min, 240 min, 300 min and 360 min. Minimum 12 samples should be analyzed, but often just 6 samples are enough especially in routine work.

Chromatographic method

Quantification was performed with an in-house developed HPLC method. For analysis on HPLC, column that is used is Zorbax SB CN 250 mm x 4.6mm i.d; 5µm maintained at 40°C. Mobile phase is consisted of mixture of ammonium phosphate buffer with octane sulfonate adjusted to pH 6.5 and acetonitrile in ratio of 25:75 (v/v). Time of analysis is 10 minutes with a flow rate from 2.0 ml/min and injection volume of 20 µL. UV detection is performed on 258 nm.

Results and discussion

The results are expressed as slopes and cumulative amount of the active substance at the end of the analysis.

With correlation coefficient 0.99, analysis on laboratory trial with 50% less active substance then test product showed that the cumulative amount of this trial is 84.40 µg/cm^2 and drug release rate is 40.79. When compared to the cumulative amount and drug release rate on test product, lower limit of 90% confidence interval is 51.85 for drug release rate and 52.23 for cumulative amount and upper limit of 90% confidence interval is 52.24 for drug release rate and 52.68 for cumulative amount.

Analysis on laboratory trial with 100% more active substance then test product showed that the cumulative amount of this trial is 365.01 µg/cm^2 and drug release rate is 175.16. When compared to the cumulative amount and drug release rate on test product, lower limit of 90% confidence interval is 222.57 for drug release rate and 226.99 for cumulative amount and upper limit of 90% confidence interval is 224.87 for drug release rate and 229.52 for cumulative amount. The correlation coefficient is 0.99.

Analysis on laboratory trial with different viscosity has correlation coefficient 0.98 and it showed that the cumulative amount of this trial is 130.73 µg/cm^2 and drug release rate is 63.66. When compared to the cumulative amount and drug release rate on test product, lower limit of 90% confidence interval is 82.81 for drug release rate and 83.11 for cumulative amount and upper limit of 90% confidence interval is 84.12 for drug release rate and 84.45 for cumulative amount.

The results are considered valid if they fulfill linearity criteria (r^2=0.90). According to EMA's Draft guideline on quality and equivalence of topical products, results from analysis should fall within the acceptance criteria from 90 - 110%. Results from all laboratory trial are out of the acceptance criteria. Laboratory trial with 50% less active substance has around 50% less cumulative amount of active substance in last sampling point and lower drug release for around 50% from the test product. Laboratory trial with 100% more active substance then the test product has around 100% higher cumulative amount of active substance in last sampling point and 100% faster drug release rate, as expected. Laboratory trial with different viscosity has also showed difference in cumulative amount of active substance in last sampling point and drug release rate in comparison with the test product.

Conclusion

Results are out of the acceptance criteria from EMA's Draft guideline on quality and equivalence of topical products, which means that this in-house developed method is sensitive to changes in formulations. Due to its discriminatory power, method for IVRT can be used for comparison of batches and quality control.

References

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