Accelerated development of pharmaceutical formulations through expeditious methodologies

Casimiro, N.[1,2]; Bica, A.[3]; Menezes, J.C.[1]; Lopes, J.A.[2]

[1]Instituto Superior Técnico da Universidade de Lisboa

[2]Faculdade de Farmácia da Universidade de Lisboa

[3]Laboratório Medinfar – Produtos Farmacêuticos, S.A

Abstract

This work aims to present a strategy for the accelerated development of generics through expeditious analytical methodologies coupled to chemometric analyses. The major objective is to unveil formulations' properties through reverse engineering, with the final aim of shortening development time and thus time-to-market. This work was based on an acyclovir ointment of commercial name Zovirax 5%. Zovirax 5% ointment, consists of 5% (w/w) acyclovir and 95% (w/w) of a polyethylene glycol base (reference product). The nature of PEGs in the composition is not disclosed in the patient information leaflet. Composition of the PEG base was unveiled by MALDI-TOF spectrometry and GC/MS. Quantification of excipients was performed resourcing to an experimental design intended to produce a set of convenient samples. Produced samples and lots from the reference product were analysed by NIR spectroscopy in diffuse reflectance mode. These spectra were pre-processed with a Savitzky-Golay filter (first derivative) and analysed by hierarchical cluster analysis. Obtained results indicate that the reference product besides acyclovir (5%) is most probably composed by PEG-300 (45±2.5% w/w) and PEG-1500 (50±2.5% w/w). The possible presence of propylene glycol suggested by the fact that it will improve incorporation of acyclovir in the PEG base was discarded. The proposed approach was not only successfully demonstrated in this case but can be effectively used for other solid and semi-solid drug products.

Keywords: Reverse engineering; Mass spectrometry; Near infrared spectroscopy; Design of experiments; Acyclovir

1. INTRODUCTION

In order to achieve bioequivalence, generics tend to copy the reference products, using information from the package leaflet, patents, product literature and reverse engineering data. The ultimate goal in the development of a generic product for topical application is to achieve qualitative equivalence (Q1) - to contain the same components as the reference product - and quantitative equivalence (Q2) - to contain the same components at the same concentration (±5%) as the reference product. There is a huge advantage in developing a formulation that presents Q1 / Q2 equivalence, because the company may in some situations be excluded from conducting in vivo bioequivalence studies. To overcome in vivo bioequivalence studies, data must support equivalence in terms of characteristics that the developed product must share with exhibits the same characteristics and

physical-chemical properties with the reference product (1–6). Nonetheless, for semisolid products, it is not required for the generic product to show Q1 / Q2 equivalence, although there is a greater regulatory inquiry into formulations which do not show this equivalence, forcing the company to demonstrate that the physico-chemical characteristics, attributes critical criteria and the rate of flow of the generic (through in vitro human skin permeation studies and/or percutaneous absorption studies in animal models in vivo) are similar to those of the reference product(4,6–10).

Through reverse engineering, all potential problems, such as product critical quality attributes, stability and effectiveness, can be minimized. Because of the patent protection or undesirable properties that may be present in the reference product formulation, the company may choose to reformulate it, in order to improve product attributes. These modifications need to be justified accordingly to their expected functionality(4,11,12).

This work explores the combined implementation of fast and expeditious analytical methods with chemometrics for the accelerated development of formulations based on reverse engineering. Qualitative analysis was investigated resourcing to MALDI-TOF spectroscopy and gas chromatography/mass spectroscopy (GC/MS). For the quantitative analysis, a series of drug product samples were planned based on an experimental design (DoE) and analysed by near-infrared spectroscopy (FT-NIR). A chemometric method (hierarchical cluster analysis) was applied to determine the components' proportions.

2. MATERIAL AND METHODS

MALDI-TOF spectrometry

The equipment used was a mass spectrometer 4800 Plus MALDI TOF/TOF™ (SCIEX, Concord, Ontario). Spectra were acquired in a positive reflector mode for the mass window of 700m/z-5000m/z. The samples were diluted in an α-cyano-4-hydroxycinnamic acid (CHCA) matrix at a concentration of 10mg/mL, dissolved in 50% acetonitrile/0.1% trifluoroacetic acid.

Mass spectrometry

Experiments were performed on a triple quadrupole mass spectrometer Micromass Quattro Micro API™ (Waters Corporation, Milford, MA), with an attached electrospray ionisation (ESI) source. The spectra were acquired in positive mode for the mass window of less than 700m/z, and the negative mode was used for the detection of propylene glycol. The samples were prepared with a concentration of 1mg/mL in acetonitrile and filtered through a 0.22µm mesh PTFE filter. Before analysis, the samples were diluted in acetonitrile until a concentration of 100µg/mL.

Gas chromatography

Samples were prepared with a concentration of 50mg/mL in methanol, adding 0.10mg/mL of 2,2,2-trichloroethanol as an internal standard and the standard solution was prepared with 2.0mg/mL of propylene glycol in methanol, adding 0.10mg/mL of 2,2,2-trichloroethanol as internal standard(13). All samples were filtered through a 0.22µm mesh PTFE filter. A ZB 5-MS, Zebron, 30m (length) x 0.25mm (internal diameter x 0.25µm (film thickness) column coupled to a flame ionization detector was used. Table 1 and

Table *2* shown the settings used in the analysis.

Table 1. Gas chromatography conditions

Parameter	Condition
Injection volume	1 µL
Carrier gas	Helium
Carrier flow	2 mL/min
Injetor temperature	250 ºC
Injection mode	Splitless (1.5 min)
Split ratio	1/50
Ionic source temperature	250 ºC
Detector temperature	250 ºC
Analysis time	16 min

Table 2. Oven program

Ratio (ºC / min)	Final temperature (ºC)	Retention time (min)
	35.0	5.00
35.00	200.0	1.00
35.00	325.0	10.00

Near infrared spectroscopy

Spectra were acquired on a FTLA2000 (ABB Inc., Québec, QC), under software control GRAMS/AI™ (Version 7.0.0, Thermo Fisher, Waltham, MA). Spectra were acquired in diffuse reflectance mode, using polytetrafluoroethylene (PTFE) as background. Solids were measured inside borosylicate flasks and a home-designed PTFE disc for liquid compounds (PEG300). Acquisition settings are shown in Table 3.

Spectra were preprocessed by Savitzky-Golay (1st derivative, filter size with 15 points and 2nd polynomial order) eliminating unwanted light scattering effects.

Table 3. Settings used to acquire NIR spectra

Parameter	Condition applied
Method	Diffuse reflection
Detector	InGaAs
Background	PTFE
Resolution	8 cm^{-1}
Scans	32
Spectral window	10000 cm^{-1} – 4000 cm^{-1}
Replicates	Triplicates

Samples preparation

The manufacturing process for producing ointment samples, was based on a protocol previously used by Medinfar[1]. The manufacturing process steps were:

1) incorporate the active substance into part of the PEG with lower molecular weight;
2) add the remaining PEG with lower molecular weight to the PEG with higher molecular weight and melt;
3) add mixture 2) to mixture 2);
4) allow to cool to room temperature and
5) pack.

Experimental design

An experimental design (DoE) was performed using Umetrics MODDE Pro (Version12, MKS Instruments AB, Umeå, Sweden).

Hirarchical cluster analysis

Hierarchical cluster analysis resourcing to the Ward's algorithm and the Euclidean distance were applied to process NIR spectroscopy data aiming at unveiling the

[1] *Note that the manufacturing process described here will be addressed in a general way, due to confidentiality issues, although this method has optimized and critical production parameters identified.*

approximate composition of the reference product.

All chemometric analyses were performed using MATLAB (Version 8.3, MathWorks, Natick, MA) and PLS Toolbox (Version 8.2.1, Eigenvector Research Inc., Wenatchee, WA).

3. RESULTS AND DISCUSSION

Qualitative analysis

Regarding the detection of propylene glycol, it was not possible to confirm the presence using mass spectrometry because the equipment used only detects from 45m/z. Since the three most intense peaks are below this value, was used gas chromatography.

Gas chromatografy

As a qualitative analysis, the presence of propylene glycol was only considered in samples with values higher than 1 part per million (p.p.m). The amount of propylene glycol was extrapolated by comparing the sample areas to a standard concentration of 600 parts per billion (p.p.b.). In batch 8072471 an amount of propylene glycol of less than 600p.p.b. This result may be a consequence of out of date ointment. In batch 8094939 no propylene glycol was detected.

Mass spectrometry

Relatively to batch 8072471 spectrum (Figure 1A), it is verified that the intensity of the set of peaks between 100m/z - 500m/z presents similar conformation to a normal distribution and the difference of molecular mass between them is constant in 44m/z, being the highest peak of this distribution at 327m/z. All data acquired on this spectrum indicates that the present PEG has a mean molecular weight of 300.

MALDI-TOF Spectromety

The intensity of the peak set of the spectrum of batch 8092267 (Figure 1B), located between 1050m/z - 2050m/z, have similar conformation to a normal distribution and the molecular weight difference between them is constant at 44m/z, with the highest peak of this distribution at 1538m/z. All data acquired on this spectrum indicates that the present PEG has an average molecular weight of 1500. Through the results obtained by mass spectrometry it is found that, qualitatively, the reference ointment, in terms of PEG base, is composed of PEG300 and PEG1500.

With the results obtained in the qualitative analysis, it was concluded that the reference ointment, at excipients level, is composed only by a PEG base formed by PEG300 and PEG1500 in the absence of propylene glycol in its constitution.

Quantitative analysis

To unveil the composition of the PEG base (PEG300 and PEG1500), a series of ointments were produced in lab scale resourcing to a DoE. The DoE varied only the constituents present in the formulation and kept constant the factors inherent to the manufacturing process (temperature, stirring speed and stage times). The acyclovir content was set to vary around 5% (w/w) with a variation of ±5%. For PEGs, which make up the remaining 95% (w/w) of the ointment, the amount of PEG1500 was centred at 50% (w/w), varying ± 10% and PEG300 was the filler. The DoE considered three factors (acyclovir, PEG300 and PEG1500), a full factorial design with two levels.

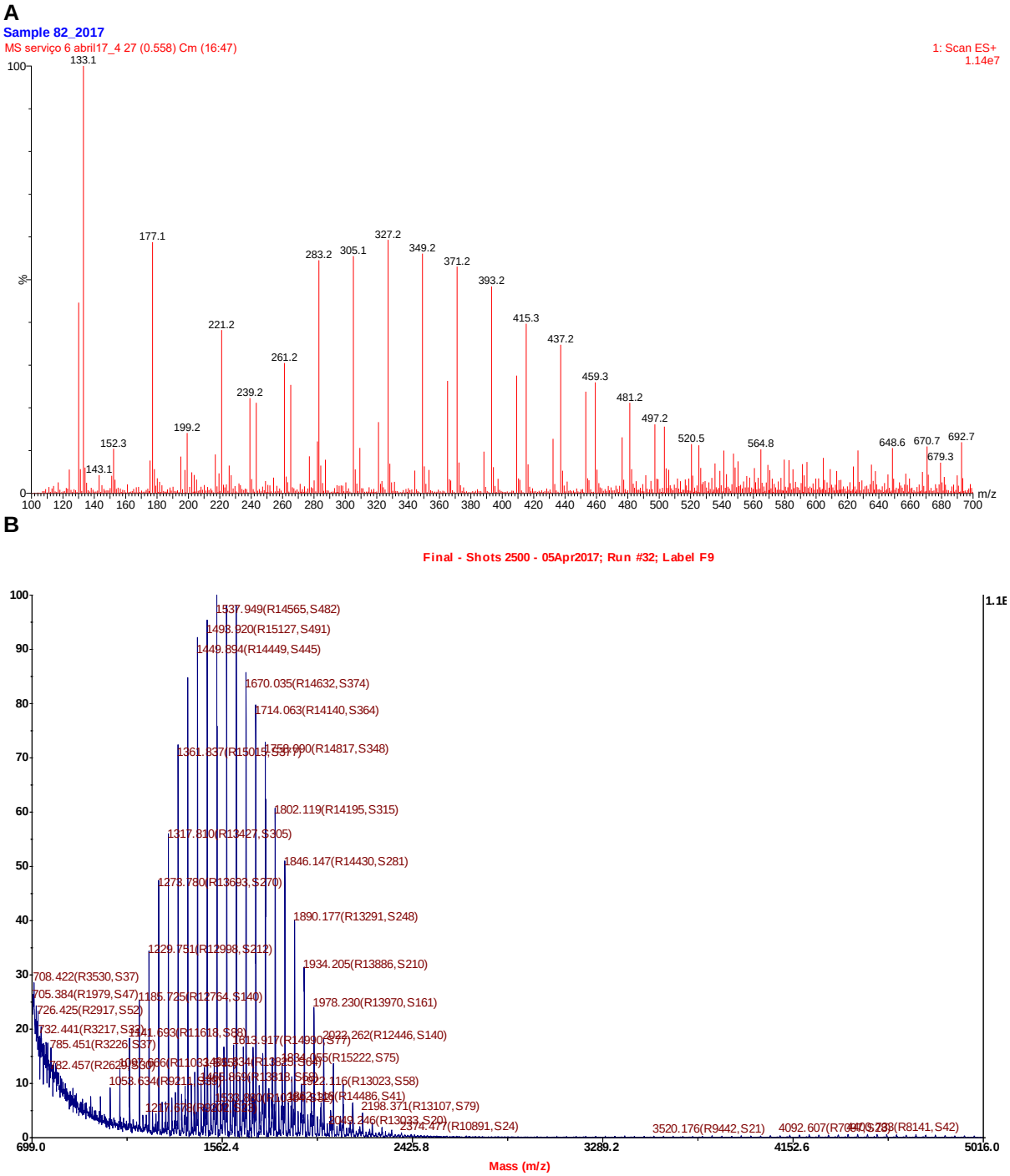

Figure 1 - Batch 8072471 mass spectrum for the 100m/z - 700m/z (A) window and batch 8092267 mass spectrum for the 700m/z - 5000m/z (B)

The DoE yielded seven experiments (four design runs and three centre points) that were performed under lab-scale conditions following a randomized order, thus avoiding systematic errors and minimizing possible disturbances that may exist during preparation.

DoE prepared samples, pure compounds and 3 commercial lots of the reference product were analysed by NIR spectroscopy. The NIR spectrum of the pure compounds revealed the expected similarity between PEG300 and PEG1500, that vary essentially in terms of peaks intensities. Acyclovir presents a very characteristic spectrum that is easily identified from the PEGs. A first analysis of NIR spectra of DoE batches and lots of the reference

product (batch 8092267) revealed acyclovir peak shifts in some areas of the spectrum. This evidence was found for the reference product spectrum and some DoE batches. The shift occurs towards lower wavenumbers by about 50cm^{-1}. Acyclovir bands that shifted were located at (4545cm^{-1}, 4790cm^{-1}, 5075cm^{-1}, 5950cm^{-1}, 6600cm^{-1}, 6825cm^{-1}).

The peak present at 4545cm^{-1} mainly correspond to interactions of N-H, C-N, C = O bonds, being also a zone of water absorption(14,15). At 4790cm^{-1}, the peak correspond mainly to N-H bond interactions present on amides and O-H bonds present in polymers (14,15). The peak at 5075cm^{-1} correspond mainly to interactions of N-H bonds present in primary amines of aromatic compounds(14,15). The peak present at 5950cm^{-1} mainly correspond to interactions of C-H bonds related to carbonyl groups(14,15). At 6600cm^{-1}, the present peak correspond mainly to interactions of N-H bonds in secondary amines(14,15). The peak at 6825cm^{-1} mainly to interactions with N-H(14,15). Since these shifts are mainly associated to acyclovir spectrum and occurring in zones of water absorption peaks, it was decided to evaluate the presence of water in acyclovir, in order to verify if at any moment of the process, through the interaction with the PEGs or due to the pack conditions, acyclovir was hydrated(16–18). For this purpose, some acyclovir was placed in an oven at 50ºC, and the spectrum was acquired after 17h and again placed in the oven, this time at 70ºC, and the spectrum was acquired after 4h. A minimum difference was observed, occurring only at the intensity level, confirming that the presence of water is not the cause of shifted peaks.

In order to test the hypothesis that peaks shifts were due to interactions between PEGs, PEG bases were prepared without acyclovir. One batch showing shifted peaks was replicated but without acyclovir. It was found that the shifted peaks were not due to interactions between the PEGs, concluding that acyclovir is responsible for the shifting. By carefully analysing the procedure used in the preparation of the samples, it was verified that the cause could be related with the amount of PEG300 used in the acyclovir incorporation. Moreover, shifted peaks might be related with the successful incorporation of acyclovir in the PEG base, thus serving as an indicator of finished product uniformity.

However, and because peaks shift distort spectral alignment, this will impact on the application of chemometric methods to these data. To overcome this problem, the spectral zones around peaks that shifted were eliminated from further analysis. The chemometric analysis rationale consisted in comparing spectra DoE batches to the reference product Zovirax resourcing to HCA.

The resulting dendrogram (Figure 2) shows that commercial batch L8092267 and DoE batch ACY122 are extremely similar. Due to the similarity between these samples it was concluded that the percentage of PEG300 and PEG1500 in the ointment is approximately 45% and 50%, respectively. More accurate estimations could be obtained with more produced samples, and applying a multivariate regression method such as PLS. However, the proposed method is extremely expeditious and provides a rather approximate composition of excipients. Since NIR spectra has a very high signal-to-noise ratio, the uncertainty in this result can be related with uncertainty in samples preparation (manufacturing process). An estimation of a ±2.5% of error for each

estimation can be considered an acceptable value. Indeed, considering this error range, it cannot be excluded that the actual PEG base can be one existing in the market composed by equal amounts of PEG-300 and PEG-1500.

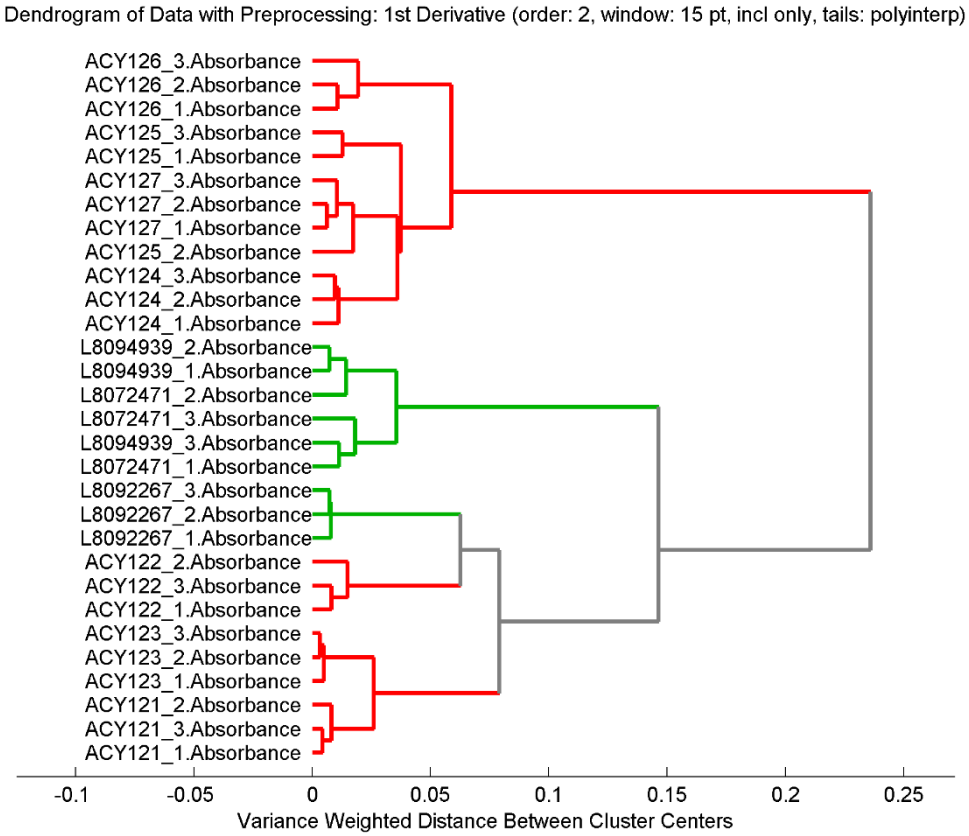

Figure 2 - Dendrogram obtained from HCA on NIR spectra of DoE samples (ACY*) and Zovirax lots (L*).

4. CONCLUSION

It has been shown that, through MS, it was possible to identify the compounds present in the reference product formulation, with PEG300 and PEG1500 being identified. Propylene glycol was not detected.

The manufacturing method used was optimized based on an already existing procedure, by identifying the critical parameters of the process.

NIR spectrophotometry showed to be quite sensitive, being able to identify differences in the microstructure (absence of total incorporation of acyclovir or absence of acyclovir). These differences were in the form of shifts towards lower wavenumbers of acyclovir peaks by around 50cm^{-1}.

NIR spectra of designed formulations around an estimated target formulation and commercial lots of Zovirax, revealed a strong similarity between batch ACY122 and Zovirax lot. It was possible to present a final formulation consisting of 5% (w/w) acyclovir, 45±2.5% (w/w) PEG300 and 50±2.5% (w/w) PEG1500.

This work was mainly focused on the formulation development through reverse engineering. In this particular case, a more intensive DoE could provide samples for the application of a quantitative method such as PLS for a more accurate quantitative assessment. It is also of interest to investigate in further detail the origin of the peak shifts

observed for acyclovir. Additionally, it is of interest to incorporate in this analysis the impact of the manufacturing process as replicating the reference product is required in this approach. The quantitative approach might be based on other methodologies, such as fluorescence, mid-infrared (ATR) and Raman spectroscopy or even a combination of them.

REFERENCES

1. Davit BM. Regulatory Approaches for Generic Drugs: BE of Topical Drug Products. In: Evaluation of New and Generic Topical Drug Products [Internet]. 2013. p. 21–4. Available from: http://pqri.org/wp-content/uploads/2015/08/pdf/Davit.pdf
2. Food and Drug Administration. Draft Guidance on Acyclovir [Internet]. 2012. Available from: https://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm296733.pdf
3. Food and Drug Administration. FDA CDER to Valeant Pharmaceuticals Intl Inc Petition Denial [Internet]. 2012. Available from: https://www.regulations.gov/document?D=FDA-2012-P-0779-0004
4. Chang R-K, Raw A, Lionberger R, Yu L. Generic development of topical dermatologic products: formulation development, process development, and testing of topical dermatologic products. AAPS J [Internet]. 2013 Jan 10;15(1):41–52. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3535108/
5. Chang R-K, Raw A, Lionberger R, Yu L. Generic development of topical dermatologic products, Part II: quality by design for topical semisolid products. AAPS J [Internet]. 2013 Jul [cited 2017 Sep 1];15(3):674–83. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23572241
6. Shah VP, Yacobi A, Rədulescu FŞ, Miron DS, Lane ME. A science based approach to topical drug classification system (TCS). Int J Pharm [Internet]. 2015;491(1–2):21–5. Available from: http://www.sciencedirect.com/science/article/pii/S0378517315005220?via%3Dihub
7. Shah VP. Topical Drug Products Regulatory Requirements (USA). In: To and Thru the Skin [Internet]. Mumbai, India; 2009. Available from: http://www.ipapharma.org/pdf/speaker/1.02 Vinod Shah topical.pdf
8. Lionberger R. Quality by Design for Topical Dosage Forms [Internet]. Available from: https://www.fda.gov/ohrms/dockets/ac/05/slides/2005-4137S2_04_Lionberger.ppt
9. Lu M, Xing H, Chen X, Xian L, Jiang J, Yang T, et al. Advance in bioequivalence assessment of topical dermatological products. Asian J Pharm Sci [Internet]. 2016;11(6):700–7. Available from: http://dx.doi.org/10.1016/j.ajps.2016.04.008
10. Yacobi A, Shah VP, Bashaw ED, Benfeldt E, Davit B, Ganes D, et al. Current challenges in bioequivalence, quality, and novel assessment

technologies for topical products. Pharm Res [Internet]. 2014;31(4):837–46. Available from: https://doi.org/10.1007/s11095-013-1259-1

11. Bansal AK, Koradia V. The Role of Reverse Engineering in the Development of Generic Formulations [Internet]. Vol. 29. 2005 [cited 2017 Sep 1]. Available from: http://www.pharmtech.com/role-reverse-engineering-development-generic-formulations?id=&pageID=1&sk=&date=
12. Food and Drug Administration. Guidance for Industry Nonsterile Semisolid Dosage Forms [Internet]. 1997. Available from: https://www.fda.gov/downloads/drugs/guidances/ucm070930.pdf
13. The United States Pharmacopeial Convention. Propylene glycol. In: United States Pharmacopeia–National Formulary [Internet]. 2010. Available from: http://www.uspnf.com/sites/default/files/usp_pdf/EN/USPNF/propyleneGlycol.pdf
14. Burns DA, Ciurczak EW. Handbook of Near-Infrared Analysis. Third Edit. Florida, EUA: CRC Press; 2007.
15. Workman J, Weyer L. Practical Guide to Interpretive Near-Infrared Spectroscopy. Florida, EUA: CRC Press; 2007.
16. Kogermann K. Understanding solid-state transformations during dehydration : new insights using vibrational spectroscopy and multivariate modelling. Universidade de Helsínquia; 2008.
17. Krzyzaniak JF, Williams GR, Ni N. Identification of phase boundaries in anhydrate/hydrate systems. J Pharm Sci [Internet]. 2007;96(5):1270–81. Available from: http://dx.doi.org/10.1002/jps.20941
18. Jørgensen AC, Airaksinen S, Karjalainen M, Luukkonen P, Rantanen J, Yliruusi J. Role of excipients in hydrate formation kinetics of theophylline in wet masses studied by near-infrared spectroscopy. Eur J Pharm Sci [Internet]. 2004;23(1):99–104. Available from: https://doi.org/10.1016/j.ejps.2004.06.001