

RESEARCH ARTICLE

A Validated IVRT Method to Evaluate Semisolid Dosage Form Containing Ximenynic Acid Using a Novel Approach

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ABSTRACT

Purpose: Ximenynic acid (XMA) holds significant potential in the market, especially due to its widespread application in the cosmetics sector. As its various biological activities continue to be discovered, there is a sharp rise in demand for ximenynic acid. A novel analytical approach has been devised for an in-house product, comparable to the reference listed drug (Softalia, containing 30% ximenynic acid), utilized in gel formulations. This method is designed to conduct *in-vitro* release studies employing Franz vertical diffusion cell apparatus and analytical quantification *via* high-performance liquid chromatography (HPLC).

Methods: The methodology has been assessed concerning specificity, linearity, the limit of quantitation (LoQ), limit of detection (LoD), inter-day precision, intermediate precision, accuracy, and solution stability. Following method development, the reference product containing 30% ximenynic acid underwent testing against the in-house XMA semisolid dosage form to accurately demonstrate “equivalence”, with results aligning with the applicable acceptance criteria falling within the range of 75 to 133.33%, as outlined in the FDA’s SUPAC-SS guidance. The *in-vitro* release assay has proven valuable for quality assurance, batch comparison, and monitoring the impacts of different formulations, scales, scaling up/down, methods, locations, and other variables.

Results: This research fabricates specific IVRT techniques for performing assessments of semisolid dosage forms, which are required for many regulatory purposes.

Keywords: Ximenynic acid, *Santalum album* L, IVRT, HPLC

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INTRODUCTION

The skin acts as a barrier between the inside of the body and the outside world. This barrier is both biological and inorganic. The conditions on the skin are perfect for the reproduction and spread of germs, including those responsible for a broad variety of illnesses. In recent years, there has been a rise in interest in using medicinal substances produced from wild plants.¹⁻⁴ Oxidative stress, caused by oxygen and nitrogen free radicals created within the body itself, may worsen the symptoms associated with aging.^{5,6} The process of getting older is a very complicated one from a biological standpoint. Wrinkling, hyperpigmentation, age spots, melasma, freckling, lentigo, ephelides, nevus, browning, and melanoma are examples of skin damage caused by reactive oxygen species.^{7,8}

Sandalwood, sometimes known as “Green Gold,” originates from the *Santalum album* L. tree in the Santalaceae

family. It has a long history of use in religious events in India as well as in traditional Chinese medicine.^{9,10} Its essential oil, also known as sandalwood oil, has been connected to several health advantages, including the ability to fight cancer, viruses, and carcinogens. It is widely used in the fragrance, massage treatment, and cosmetics sectors. Compounds such as tricycloekasantal, -sangallo, -standalone, -santalalean, and -curcumene have been identified as being present in the plant. Sangallo is another component that has been isolated from the plant. Both santalbic acid and stearolic acid (9-octadecynoic acid) may be found in the dark crimson, viscous oil that is produced by the *S. album* seed.^{4,9} These two acids can also be referred to as ximenynic acid. Ximenynic acid, which is also known as octadeca-11-trans-en-9-ynoic acid,¹¹ is one of the extremely uncommon acetylenic fatty acids that may be found in greater concentrations in plant seed oils. There are a wide

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variety of biological and medical applications for ximenynic acid.^{12,13} Some of these applications include antibacterial, antifungal, and anti-inflammatory characteristics. The top portions of the *Santalum* plant include minerals, flavonoids, glycosides, terpenes, acids, and tannins, while the seed oil contains vitamins, steroids, and lipids. Other components of the plant include terpenes. It is well known that the leaves, stems, and seeds of these plants may be used in the preparation of medical and cosmetic products and in the kitchen.

Research and development (R&D) has found that using *in-vitro* release testing (IVRT) is one of the most helpful strategies for examining how medications are distributed. The majority of people assume that IVRT may be used instead of bioequivalence studies when making post-approval alterations to a product,¹⁴⁻¹⁶ however, this is not the case. In addition to that, it has been utilized in the process of determining how manufacturing procedures might be improved. There are a lot of other benefits to using the IVRT programme for product development, such as figuring out which production parameters are the most important, predicting how the product will operate *in-vivo*, and examining how the product will perform overall, including how consistent the batch numbers will be.^{17,18} The USP came up with the IVRT as an alternate method, and the USP general section outlines other instruments and methodologies that may be used to carry out such evaluations. The *in-vitro* release testing procedure is essential for the pharmaceutical sector to use to establish whether or not the product in question is identical to the subject test product previously sanctioned by the FDA.¹⁹ This complies with the scale up and post approval adjustments (SUPAC) guidance of making adjustments to the process at any point during the product's lifespan without affecting its quality.

The rising demand for ximenynic acid calls for the development of innovative approaches to the chemical's production. The major goals of this endeavor are to isolate and purify the compound known as ximenynic acid and establish its identity. The other objective of this study is to figure out how to use ximenynic acid in a semisolid dose form in an IVRT method that makes use of a device called a Franz diffusion cell. Next to this method, you will see displays of the tools, methods, and outcomes that were previously reported. Suppose an *in-vitro* release approach is going to be employed in the manufacturing of generic goods. In that case, the dissolving conditions of that technique need to be sensitive enough to differentiate between even the most minute formulation or manufacturing differences. Calculating the quantity of active compounds generated in the dissolving test must be done in a way that is accurate, reliable, selective, and reproducible.²⁰⁻²²

MATERIAL AND METHODS

Experimental Materials and Reagents

Since the ximenynic acid given by Sami Lab India was already very pure (99.12%), it did not need any further purification before being used as the benchmark. The American Corporation Sigma Aldrich was used to acquire the testing and diagnostic materials consisting of dibasic sodium phosphate,

methanol, ethanol, sodium chloride, potassium chloride, monobasic potassium phosphate, and orthophosphoric acid. These chemicals were tested. In order to get the water ready for the stock solution, a French Millipore system was used to filter it. In order to carry out the diffusion study, 0.45 micro meters of PVDF membrane was acquired from Durapore EMD Millipore in Mumbai, India. Merck Ltd. in India obtained methanol and many other liquids to incorporate them into diagnostic compounds.

Ximenynic Acid Semisolid Formulation

For research purposes, a semisolid ximenynic acid cr me was developed by combining ximenynic acid that had been extracted with a variety of different excipients. Researchers investigated whether or whether a topical lotion containing ximenynic acid had anti-aging properties.^{23, 24} The investigation and clinical practices that would one day be known as IVRT were guided by the same fundamental principle.

IVRT Method Development and Validation

HPLC analysis of ximenynic acid

The equipment used for high-performance liquid chromatography (HPLC) was a Waters 2695 Alliance system, one of the Alliance product line from Waters. It was attached to a C18 column with a measurement of 4.6 by 250 mm and a particle size of 5, as well as a 2489 UV-visible detector and a 2996 PDA detector. The maximum capacity of the sample channel for the auto-sampler injection device was 10 μ L. The isocratic operation in a mobile phase comprising 60% methanol (solvent-A) and 40% water (solvent-B) was completed in 30 minutes. The mobile phase flow rate was kept constant at 1-mL minute⁻¹ for the length of the research. To ensure that the attendees are appropriately prepared. That's when the initial injection was administered. The temperature of the sample and the column were kept at 10 C for the duration of the experiment. The chromatogram was produced after the PDA's wavelength was changed to produce the strongest response from a single peak at 229 nm. By comparing the retention times and spectra of the sample solution and the standard solution, we were able to identify the standard XMA. The room temperature used for this investigation was 25 C.

In-vitro release testing of semisolid formulation

The amount of medication that was released *in vitro* from XMA cream was determined using Vertical Franz diffusion cells with an assay volume of 7 mL. The release test used an artificial/simulated tear fluid with a pH of 7.4 and was composed of 2.0% SDS, 0.0067% NaCl, 0.002% NaHCO₃, and 0.0000604% CaCl₂. After bringing the solution to a temperature of 37.5 C, new medium was added to the chamber containing the receptors. Approximately half an hour before beginning the analysis, a pre-serilized Nylon-66 Membrane Disc Filter (MDI , 0.45 m pore size) was placed in STF solution and allowed to soak for the whole 30 minutes. After each Franz diffusion cell was supplied with a saturated membrane, a white silicon ring, a riveted glass cell, and a metal cell ring, the components of the cell were then firmly clamped in place. The

Franz diffusion cells were shaken at a rate of 500 revolutions per minute. During the first, second, third, fourth, and fifth hours, 1.5 mL samples were taken automatically every hour. These samples were collected automatically. The size of the samples is determined algorithmically, depending on the total quantity of material that has been played on each device. The samples that were produced as a consequence were analyzed using the HPLC apparatus at a wavelength of 229 nm.

XMA Solubility and Receptor

The process of evolution by itself Researchers investigated several fluids that bind to receptors, including water at a concentration of 100%, water and ethanol at a volume ratio of 80:20, salt at a concentration of 0.9%, and sodium chloride at a concentration of 0.09%. The solubility of XMA in various receptor fluids was examined three times, and each time, a saturated solution was created by dissolving 200 mg of XMA in 10 mL of the respective receptor fluid. The solution was stirred at 600 rpm for 6 hours and left alone at $32 \pm 1^\circ\text{C}$ for the night. Aliquots of the supernatant were obtained, filtered, and reduced before being examined using HPLC in order to determine the amount of XMA that was successfully dissolved. The maximum amount of the medication anticipated to be absorbed by the receptor should dissolve more than 10 times when exposed to the medium.

Membrane Screening

Several synthetic membranes were used for membrane screening, including 0.45 μm Nylon filter (Cat. No. PT0901) from MDI, 0.45 μm PVDF filter (P/No. PV25045) from Maxsil, and 0.45 μm PTFE filter (P/No. PT25045). Maxsil. Individual membranes ($n = 3$) were submerged in 10 mL of a 0.9% sodium chloride solution containing XMA for six hours at a temperature of $32 \pm 1^\circ\text{C}$ in order to study the binding of XMA to the membranes. As a control, the test solution generated in duplicate but without any submerged membrane was allowed to equilibrate for 6 hours at $32 \pm 1^\circ\text{C}$. The HPLC technique outlined above was used to determine the XMA concentrations in all of the solutions. By comparing each test solution to the control solution, recoveries for each solution containing the membranes were computed. The average recovery rate for each membrane should not exceed $\pm 5\%$.

Sampling Duration

The duration of the IVRT run as well as the amount of time that passed between samples were both subject to change. There were three departures every half hour for the first two hours, and then there was one departure per hour for the next eight hours. The sample period that had the appropriate correlation, as well as recovery ($\leq 30\%$), was selected on the basis of the concepts presented by Higuchi. Samples were obtained at least six different times during the process.

IVRT Method

The IVRT employed six VDCs in parallel to maintain a temperature of $32 \pm 1^\circ\text{C}$ while agitating the receptor medium at a pace of 500 rpm throughout each cycle. Following a half-hour period for the VDC system to reach equilibrium at $32 \pm$

1°C , a degassed solution containing 0.09% sodium chloride was subsequently introduced into the receptor chambers. Following a 30-minute presoaking period in the membrane receptor media, the cream was carefully applied to the membrane surface and weighed around 300 mg. Throughout the 90-minute duration, 200 μL aliquots were taken from the receptor chamber of each of the six VDCs at regular intervals of 15 minutes. Then, we added 220 μL of receptor medium to the VDCs again. After removing the aliquots from the mixture and replacing all of the receptor medium for the VDCs, the agitation was done again. We made sure that there was a maximum of fifteen minutes of rest in between each agitation session. The HPLC method was employed to perform the aliquot analysis.

Validation of the IVRT Method

The approach described by Tiffney *et al.* was the one that was utilised to show the IVRT system's effectiveness. Creams containing XMA were used in an experiment to determine the sensitivity, specificity, and specificity of the IVRT procedure. Analyzing the release rates of moisturizers having varied concentrations of XMA allowed for the determination of the method's level of sensitivity. We evaluated the specificity of the approach by analyzing the association between the XMA release rate and the XMA concentration in the test creams. The box and whisker plot was used in the computation of the coefficient of determination (R^2). It is possible that the methodology may be proved to be correct if there is a linear link between formulations of varied strengths. A statistical method for assessing product "sameness" was used to every piece of data, as described in chapter 1724 of the United States Pharmacopoeia. In order to assess how rapidly the IVRT method might release the drug, test creams containing varied amounts of XMA were applied to the skin. In order to assure uniformity, accuracy, and consistency, the XMA release rates that were achieved in each of the three individual IVRT experiments were compared to one another. The impacts of two different stirring rate adjustments in relation to the typical stirring rate of 600 rpm were investigated, as were the effects of two different temperature adjustments ($2 \pm 2^\circ\text{C}$) in comparison to the nominal temperature of 32°C (at $32 \pm 1^\circ\text{C}$)²³⁻²⁴.

RESULTS

IVRT Method Development and Validation

Receptor medium selection and sampling qualification

A random selection resulted in the use of six diffusion cells, each of which included six cells. These cells were then treated with the XMA test product, and the receptor media was analyzed hourly for the first eight hours following the first dose. Each diffusion cell was fastened to a single kind of nylon synthetic membrane that measured 0.45 μm in thickness and 25 mm in length. Each of the four distinct receptor solutions is evaluated on a total of six different cells (Tables 1-17). The following criteria must be satisfied by the apparatus used in the diffusion cell,

Apparatus : Six diffusion cells per sample run

Surface area : 1.66 cm²
 Cell volume : 12 mL
 Sampling intervals : 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 hours
 Temperature : 32 ± 0.5°C
 rmp : 600
 Application method : Using syringes on open cap assembly
 Application amount : About 1000 mg
 Sample aliquot : 200 µL
 Membrane : PVDF, 0.45 µm, 25 mm (MDI)
 Receiving medium : 1) Water 100%
 2) Water: Ethanol: 80:20 v/v
 3) 0.9% Saline 4) 0.09% NaCl

In the examination of the sensor medium, water was the sole substance employed. It was found that a small amount of XMA was escaping up until a time point that was six hours in the past. Because it was discovered that water greatly slowed down the drug release process, research was carried out in which 20% ethanol was added to the diffusion medium to hasten the medication's release. Even though the slopes RSD were within the permissible limit at 5.92% when water and ethanol were mixed at a ratio of 80:20 (vol/vol), the percentage of drug release was discovered to be slightly larger, around 9%.

This was the case even though the slopes RSD were within the permitted limit. After that, an experiment was carried out to see whether or not utilizing a sodium chloride solution of 0.09% would improve medication delivery. Up to the six-hour mark, it was found that this diffusion medium had released about 24.08% of the test product, with a slope RSD of 5.28. When 0.9% seawater was used as the receptor medium for up to 6 hours, it was discovered that slopes RSD were within the permitted limit (5.19%); nevertheless, drug release was found to be 43.09%. This was the case despite the fact that drug release was measured.

After five hours, the sodium chloride solution with 0.09% concentration demonstrated the least amount of drug release (less than 30%) compared to the other four media that were evaluated. In every cell, the regression coefficient was higher than 0.90, and the slope relative standard deviation (RSD) was

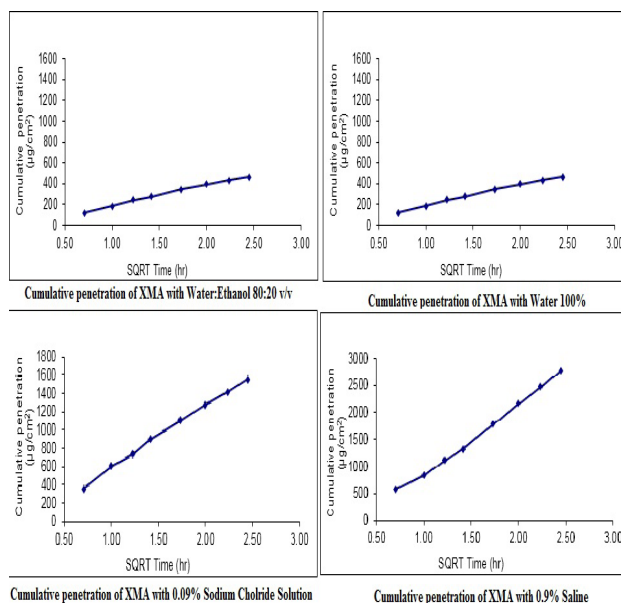


Figure 1: Cumulative penetration of XMA with different receptor media & nylon as membranes, 0.45 µm, and 25 mm

found to be lower than 15% of the limit. The results associated with the performance of the IVRT method and XMA solubility led to the conclusion that a solution of 0.09% sodium chloride would make an appropriate receptor medium. This solution would result in the release of no more than 30% of the drug at the terminal time point (selected up to 5 hours), and it would require a membrane filter with a pore size of 0.45 HVE/PVDF, 25 mm.

Membrane Filter Selection

In this experiment, a potential receptor medium will be dosed onto an RLD-strength test product placed on one of four distinct types of synthetic membrane filters placed in six diffusion cells. The experiment will be carried out in order to determine whether or not the receptor medium is effective. The tentative study design was summarized as,

Apparatus : Six diffusion cells per sample per run
 Surface area : 1.66 cm²

Table 1: Slopes and regression coefficient value of XMA with different receptor media & PVDF as membranes, 0.45 µm, and 25 mm

Sr: No	Water 100%		Water: Ethanol 80:20 v/v		0.09% Sodium chloride solution		0.9% Saline	
	Slopes	Regression coefficient	Slopes	Regression coefficient	Slopes	Regression coefficient	Slopes	Regression
1	203.40	0.998	255.15	0.992	656.59	0.994	1313.50	0.992
2	185.29	0.993	222.80	0.995	659.79	0.996	1261.48	0.993
3	189.60	0.993	246.69	0.992	627.27	0.998	1170.90	0.994
4	206.99	0.996	232.02	0.996	721.08	0.998	1359.54	0.994
5	231.11	0.993	261.81	0.995	702.08	0.997	1320.06	0.999
6	180.16	0.987	244.44	0.995	702.63	0.997	1324.64	0.997
Mean	199.42	0.993	243.82	0.994	678.24	0.997	1291.69	0.995
SD	18.68	*	14.43	*	35.79	*	67.04	*
%RSD	9.37	*	5.92	*	5.28	*	5.19	*

* %RSD calculated for slopes only

Sampling intervals : 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 hours
 Temperature : 32°C ± 0.5°C
 rpm : 600
 Application method : Open Cap Assembly
 Application amount : about 1000 mg
 Sample aliquot : 200 µL
 Membrane filters : 0.45 µm Nylon, 0.45 µm PVDF, 0.45 µm PTFE.
 Receiving medium : 0.09% NaCl solution

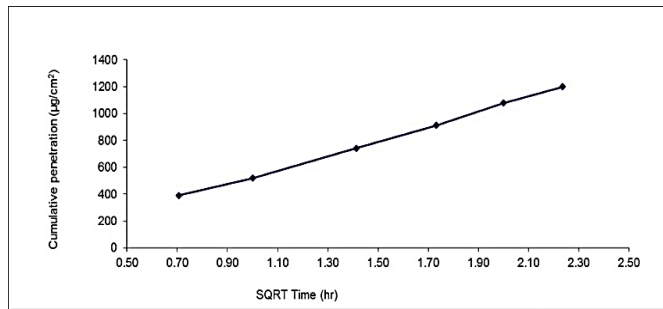
Screening of all three membrane filters done using above IVRT conditions and results of all the membranes has been tabulated as follows;

According to the study conducted on membrane selection, the total entrance release profile of XMA from XMA cream employing 0.45 µm nylon, 0.45 µm PVDF, 0.45 µm PTFE Membrane, 0.45 and 25 µm membranes was linear up to 5 hours of time points. This was the case with all of the membranes tested. The linear regression coefficient (r²) and relative standard deviation (%RSD) of slopes for the 0.45 PVDF membrane fell within the same range as those for the other two membranes.

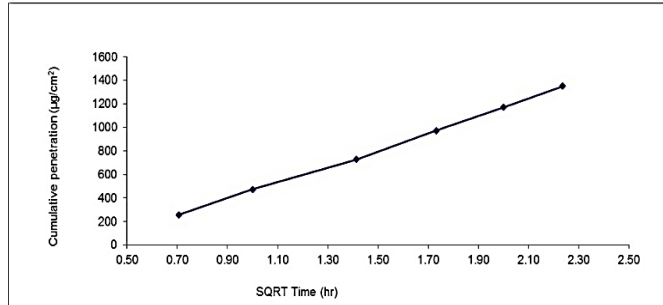
Selection of Sampling Time Points

A dosage of receptor solution will be administered to the test product, and samples will be collected every half hour for the first two hours, followed by taking samples every hour for the following eight hours. These samples will be obtained from six diffusion cells, each of which has six cells, and they will be put on a random kind of PVDF synthetic membrane.

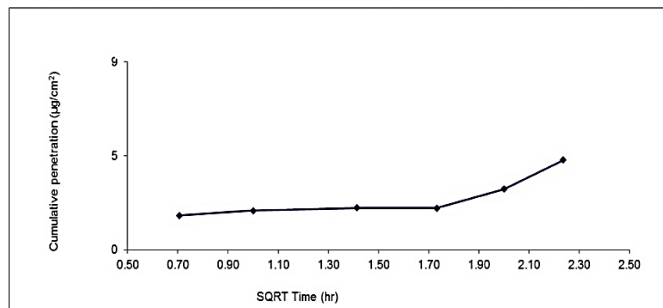
Apparatus : Six diffusion cells per sample run
 Surface area : 1.66 cm²
 Cell volume : 12 mL
 Sampling intervals : 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 hours
 Temperature : 32 ± 0.5°C
 rpm : 600
 Application method : Using syringes on open cap assembly



Graph of cumulative penetration of XMA with Nylon 0.45µm, 25 mm membrane



Graph of cumulative penetration of XMA with PVDF 0.45µm, 25 mm membrane



Graph of cumulative penetration of Clindamycin with PTFE 0.45µm, 25 mm membrane

Figure 2: Graph of cumulative penetration of XMA with 0.45 µm nylon, 0.45 µm PVDF, 0.45 µm PTFE Membrane

Application amount : About 1000 mg
 Sample aliquot : 200 µL
 Membrane : PVDF, 0.45 µm, 25 mm (MDI)
 Receiving medium : 0.09% NaCl in water

Table 2: Results of cumulative penetration of XMA with 0.45 µm Nylon, 0.45 µm PVDF, 0.45 µm PTFE Membrane, 0.45 µm and 25 mm

0.45 µm Nylon		0.45 µm PVDF		0.45 µm PTFE	
Square root of time	Average cumulative penetration (µg/cm ²)	Square root of time	Average cumulative penetration (µg/cm ²)	Square root of time	Average cumulative penetration (µg/cm ²)
0.71	390.65	0.71	256.24	0.71	1.73
1.00	519.61	1.00	472.54	1.00	1.98
1.41	742.20	1.41	727.30	1.41	2.12
1.73	911.93	1.73	973.22	1.73	2.10
2.00	1078.19	2.00	1170.07	2.00	3.07
2.24	1199.71	2.24	1351.71	2.24	4.53
Average r ²	0.998		0.999		0.562
Average slope (µg/cm ² /hr ^{1/2})	536.526		710.950		1.493
% RSD of slope	5.08		4.76		241.59

Table 3: Cumulative drug release of XMA at sampling time points up to 5 hours

Time points (Hours)	Cumulative drug release ($\mu\text{g}/\text{sq.cm}$)
0.50	390.65
1.00	519.61
2.00	742.20
3.00	911.93
4.00	1078.19
5.00	1199.71

Table 4: Cumulative drug release of XMA at sampling time points up to 8 hours

Time points (Hours)	Cumulative drug release ($\mu\text{g}/\text{sq.cm}$)
1.00	513.10
2.00	735.69
3.00	905.42
4.00	1071.68
5.00	1193.20
6.00	1301.40
7.00	1329.17
8.00	1356.39

HPLC Method Suitability, Linearity, Accuracy and Specificity Study

The HPLC analysis technique was applied to assess the collected IVRT sample. In order to establish whether or not the approach could be used, its linearity, accuracy, and specificity were put to the test.

Linearity

We were able to determine the linearity by injecting XMA standard solutions at concentrations ranging from 2.5 to 492 $\mu\text{g}/\text{mL}$ while the HPLC was operating under the parameters

Table 5: Peak areas and XMA concentrations

S. No.	Concentration of XMA (ppm)	Response
S1A	2.46	5060
S2A	24.65	50455
S3A	49.29	102045
S4A	123.24	253467
S5A	246.47	507343
S6A	320.41	653471
S7A	419.00	855134
S8A	492.95	996208
Slope		2028.33261
Y-Intercept		2333.05983
Correlation coefficient		0.999960

LINEARITY OF CLINDAMYCIN PHOSPHATE IVRT METHOD

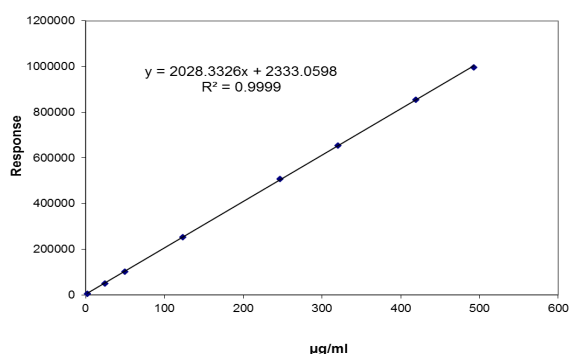


Figure 3: Graph of peak areas versus XMA

described above. An investigation into how the newly formed peak areas' linearity is affected by the sample's concentration.

The cumulative release of XMA inside the IVRT method's stated range is shown in the accompanying linearity graph, and the approach is adequately linear across the supplied range.

Table 6: Table for accuracy

Level/Sample No.	Amount added (μg)	Amount recovered (μg)	% Recovery	Mean recovery
Level-1_2.5 ppm_Prep-1	2.6621	2.7807	112.75	
Level-1_2.5 ppm_Prep-2	2.6621	2.9127	118.30	113.76
Level-1_2.5 ppm_Prep-3	2.6621	2.7103	110.11	
Level-2_50 ppm_Prep-1	53.3241	53.8364	109.01	
Level-2_50 ppm_Prep-2	53.3241	54.5493	104.78	109.72
Level-2_50 ppm_Prep-3	53.3241	53.8711	109.11	
Level-3_245 ppm_Prep-1	266.3442	267.5477	108.35	
Level-3_245 ppm_Prep-2	266.3442	267.5438	108.35	108.24
Level-3_245 ppm_Prep-3	266.3442	267.5255	108.35	
Level-4_490 ppm_Prep-1	533.9321	525.9117	106.78	
Level-4_490 ppm_Prep-2	533.9321	525.7405	106.77	106.92
Level-4_490 ppm_Prep-3	533.9321	526.7501	106.92	
Overall Mean			109.72	
Overall SD			3.192	
Overall %RSD			3.15	

Table 7: Cumulative %drug release of XMA in XMA cream for method precision

Time points	0.5 hour	1.0 hour	2.0 hours	3.0 hours	4.0 hours	5.0 hours
SPL 1	6.51	9.19	15.23	18.18	21.07	23.35
SPL 2	6.42	9.37	15.37	18.43	21.60	23.93
SPL 3	5.65	8.00	13.81	16.92	20.18	22.36
SPL 4	7.01	10.18	15.89	18.77	22.10	23.57
SPL 5	6.93	10.02	15.68	18.52	21.86	23.35
SPL 6	6.53	9.84	15.52	19.01	22.02	24.24

Table 8: Table for slopes and regression coefficient of method precision

Sample	Slopes of method precision	Regression coefficient of method precision
1	725.367	0.997
2	744.324	0.998
3	722.198	0.997
4	713.699	0.996
5	716.320	0.996
6	757.885	0.999
Mean	729.965	0.997
SD	17.42	-
%RSD	2.39	-

NA- Mean and %RSD reported for slopes only.

Accuracy (Recovery)

Volunteers were given injections of XMA drug material at doses of 2.5, 50, 245, and 490 ppm for a period of 5 hours before the placebo of XMA cream was collected and created. The collection of area counts was an integral part of the chromatographic analysis performed on each sample solution.

The fact that the mean recovery for XMA was found to be 101.5%, with a relative standard deviation of 2.91% for concentrations ranging from 2.5 to 490 ppm, demonstrated that the HPLC technique was able to accurately identify the amount of XMA in XMA cream.

Specificity

During an IVRT study, a placebo was used instead of the active component, which allowed the researchers to assess the specificity of the HPLC technique used to analyze XMA cream. The sample that was tagged Blank was obtained five hours into the IVRT experiment, and the researchers searched for a peak in the XMA retention time. The specificity of the HPLC method for measuring XMA in IVRT sample aliquots was shown by the absence of peaks in either the placebo or the blank sample aliquots throughout the retention duration of XMA.

Method precision

Following the steps outlined in the methodology section, the IVRT method was used to prepare six different XMA Cream sample preparations, which were then injected into the HPLC. The relative standard deviation (RSD)% demonstrates that the method's accuracy is adequate. Additionally, it was discovered

Table 9: Table for Drug Release of XMA in (%) for ruggedness

Time points	0.5 hour	1.0 hour	2.0 hours	3.0 hours	4.0 hours	5.0 hours
SPL 1	3.74	6.42	12.13	15.16	18.58	20.43
SPL 2	5.61	8.41	14.20	18.13	20.79	23.91
SPL 3	4.92	7.85	13.27	16.60	20.21	23.91
SPL 4	5.48	8.67	14.76	19.11	21.84	24.01
SPL 5	5.90	9.12	15.60	19.59	23.27	24.88
SPL 6	6.14	8.81	14.89	18.32	22.31	24.42

Table 10: Table of comparison of slopes for ruggedness and method precision for XMA

Sample	Analyst -1 (Precision) Slopes of profile	Analyst -2 (Ruggedness) Slopes of profile
1	725.367	718.912
2	744.324	772.255
3	722.198	780.055
4	713.699	801.261
5	716.320	833.483
6	757.885	791.426
Individual Mean	729.965	782.898
Individual SD	17.42	37.91
Individual % RSD	2.39	4.84
Overall Mean	756.432	
Overall SD	39.437	
Overall % RSD	5.21	

Table 11: Table for regression coefficients of xma from method precision and ruggedness

Cell No.	Regression coefficient of precision	Regression coefficient of Ruggedness
1	0.997	0.997
2	0.998	0.998
3	0.997	0.995
4	0.996	0.997
5	0.996	0.996
6	0.999	0.997

that the regression coefficient for each cell was higher than 0.90, which is a significant finding. The diffusion cell technique HPLC *in-vitro* release test (IVRT) profile for XMA in XMA cream is trustworthy as a result of this.

Ruggedness (Intermediate Precision)

On separate days, a reference preparation and six samples of the same XMA cream were produced using IVRT procedures. However, the samples were injected into a different HPLC from the one that was used in the method precision.

The relative standard deviation (RSD) of the slopes of the first six roughness results is 4.84%, and the RSD of the slopes of the whole set of twelve findings is 5.21%. Because the slope regression result for each diffusion cell is more than 0.90, it

Table 12: Robustness results with respect to decreased stirring (500 rpm) and control stirring (600 rpm)

Condition	Control stirring (600 rpm)		Decrease stirring (500 rpm)	
Parameter	Slope ($\mu\text{g}/\text{cm}^2/\text{hr.}$)	Regression coefficient	Slope ($\mu\text{g}/\text{cm}^2/\text{hr.}$)	Regression coefficient
1	725.367	0.997	710.126	0.999
2	744.324	0.998	733.276	0.999
3	722.198	0.997	712.240	0.999
4	713.699	0.996	753.247	0.999
5	716.320	0.996	784.144	0.998
6	757.885	0.999	787.226	0.999
Mean	729.965	0.997	746.710	0.999
SD	17.42	NA	34.04	NA
%RSD	2.39	NA	4.56	NA

NA- Not Applicable

Table 13: Robustness results with respect to increased stirring (700 rpm) and control stirring (600 rpm)

Condition	Control stirring (600 rpm)		Increased stirring (700 rpm)	
Parameter	Slope ($\mu\text{g}/\text{cm}^2/\text{hr.}$)	Regression coefficient	Slope ($\mu\text{g}/\text{cm}^2/\text{hr.}$)	Regression coefficient
1	725.367	0.997	694.034	0.999
2	744.324	0.998	753.448	0.998
3	722.198	0.997	717.371	0.998
4	713.699	0.996	784.036	0.998
5	716.320	0.996	791.092	0.998
6	757.885	0.999	779.765	0.998
Mean	729.965	0.997	753.291	0.998
SD	17.42	NA	39.69	NA
%RSD	2.39	NA	5.27	NA

NA- Not Applicable

may be concluded that the approach is precise and trustworthy. Therefore, the IVRT examination for XMA in XMA cream utilizing the diffusion cell method and the methodology of HPLC may be repeated on several occasions.

Robustness with respect to stirring (± 100 rpm)

By comparing the release rates at 100% dose strength when the receiving media was spun at faster (700 rpm) and slower (500 rpm) speeds to those under the beginning conditions (600 rpm), the degree of resilience was assessed throughout the experiment. This was carried out to assess the material's resistance to the alteration in circumstances. The scores and regression values of the two run-related variables are displayed below. The next figure is a bar chart that shows how much XMA, on average, each of the six cells produced in response to the previously listed possible outcomes.

In order to pass the robustness tests for accuracy, the percentage of relative standard deviation (RSD) for the slopes of the six cells with the beginning circumstances and the altered conditions must be less than 15.0%. The criteria stipulate that the regression value for each cell must be lower

Table 14: Comparison of cumulative XMA penetration at three distinct churning speeds: control (600 rpm), increased (700 rpm), and decreased (500 rpm).

Time ($\text{hr}^{1/2}$)	Average cumulative penetration ($\mu\text{g}/\text{cm}^2$)		
	Control (600 rpm)	Decreased stirring (500 rpm)	Increased stirring (700 rpm)
0.71	392.38	345.42	338.32
1.00	575.28	579.65	516.89
1.41	935.54	880.88	857.74
1.73	1135.04	1134.66	1117.22
2.00	1344.38	1329.97	1306.58
2.24	1486.29	1479.16	1459.94
Average r2	0.997	0.999	0.998
Average Slope ($\mu\text{g}/\text{cm}^2/\text{hr}^{1/2}$)	729.965	746.710	753.291

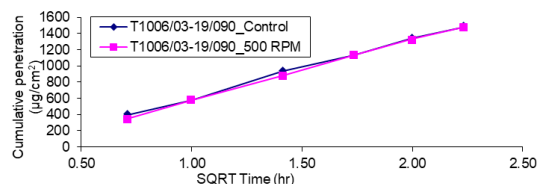


Figure 4: A graph of average cumulative amounts of XMA released with decreased stirring (500 rpm) and control stirring (600 rpm)

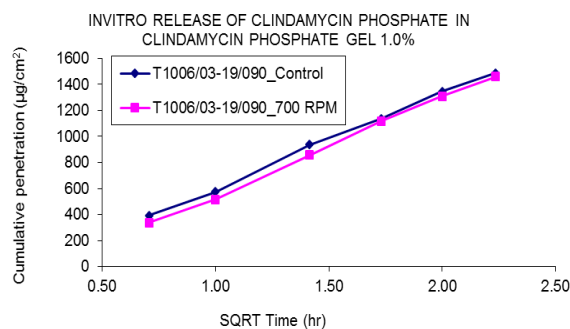


Figure 5: A graph of average cumulative amounts of XMA released with increased stirring (700 rpm) and control stirring (600 rpm)

than 0.90, and it applies to both positive and negative values. This result fulfilled the criteria.

Robustness with temperature ($\pm 1^\circ\text{C}$ at 31°C and 33°C)

By comparing the release rates from 100% dosage strength, or 1%, under the original method conditions to the release rates from 100% dose strength, or 1%, at 31°C & 33°C out temperature throughout the experiment, the resilience with regard to temperature at 31°C & 33°C was ascertained. The goal of doing this was to ascertain the ideal temperature.

The criteria for the robustness trials specify that the percentage relative standard deviation (RSD) for the slopes of the six cells under both the original and modified conditions should not surpass 15%, while the regression coefficient for each cell should be at least 0.90. Furthermore, the percentage RSD for the slopes of the three cells should not exceed 5%.

Table 15: Robustness data with respect to decreased temperature at 31°C and control temperature (32°C)

Condition	Temperature at 32°C (Control)		Temperature at 31°C	
Parameter	Slope ($\mu\text{g}/\text{cm}^2/\text{hr.}$)	Regression coefficient	Slope ($\mu\text{g}/\text{cm}^2/\text{hr.}$)	Regression coefficient
1	725.367	0.997	703.157	0.998
2	744.324	0.998	742.510	0.999
3	722.198	0.997	723.943	0.999
4	713.699	0.996	712.263	0.997
5	716.320	0.996	715.927	0.998
6	757.885	0.999	725.390	0.999
Mean	729.965	0.997	720.532	0.998
SD	17.42	NA	13.49	NA
%RSD	2.39	NA	1.87	NA

NA: Not Applicable

Table 16: Robustness data with respect to increased temperature at 33°C and control temperature (32°C)

Condition	Temperature at 32°C (Control)		Temperature at 33°C	
Parameter	Slope ($\mu\text{g}/\text{cm}^2/\text{hr.}$)	Regression coefficient	Slope ($\mu\text{g}/\text{cm}^2/\text{hr.}$)	Regression coefficient
1	725.367	0.997	773.389	0.997
2	744.324	0.998	746.496	0.999
3	722.198	0.997	694.622	0.999
4	713.699	0.996	743.762	0.999
5	716.320	0.996	702.983	0.996
6	757.885	0.999	706.967	0.996
Mean	729.965	0.997	728.036	0.998
SD	17.42	NA	31.09	NA
%RSD	2.39	NA	4.27	NA

NA- Not Applicable

DISCUSSION

The established HPLC technique, validated for analyzing XMA in IVRT samples following ICH guidelines, met all predefined acceptance criteria for validation parameters. It demonstrated selectivity, linearity, accuracy, precision, robustness, and sensitivity with an LLoQ and sample stability for seven days. Despite not meeting the specified manufacturer’s criteria for VDC volume, the VDCs proved to be precise and suitable for IVRT experiments. Thus, subsequent IVRT experiments utilized these cells, with calculations adjusted accordingly. XMA quantity was determined using a rapid, straightforward, and selective HPLC method that did not require false or blank samples. This established technique for *in-vitro* release determination, separation, and quantification of XMA in gel formulations is highly selective, reproducible, precise, and rapid, ensuring high reliability. The IVRT technique for measuring XMA by HPLC was found to be specific, sensitive, selective, robust, and reliable based on method improvement and validation investigations, making it suitable for its intended

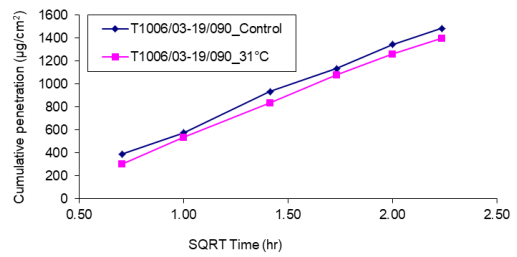


Figure 6: Graph comparison of cumulative penetration of XMA at decreased temperature and control temperature

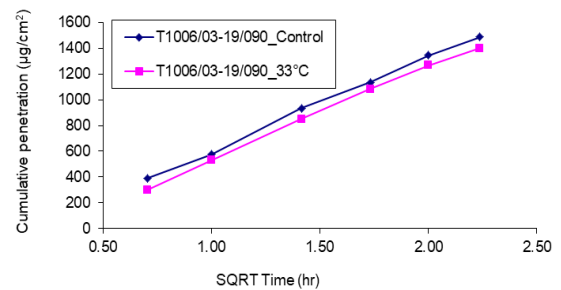


Figure 7: Graph comparison of cumulative penetration of XMA at increased temperature and control temperature

Table 17: Comparison of cumulative penetration of XMA at different temperatures

Time (hr/2)	Average cumulative renetration ($\mu\text{g}/\text{cm}^2$)	At 32°C (Control temperature)	At 31°C (Low temperature)	At 33°C (High temperature)
0.71	423.38	328.83	323.42	323.22
1.00	621.67	578.72	572.43	573.62
1.41	1010.99	900.42	913.07	919.17
1.73	1227.61	1167.87	1169.32	1169.88
2.00	1453.67	1357.72	1367.31	1369.59
2.24	1605.33	1508.04	1516.95	1517.86
Average r2	1.0796	0.9799	0.9798	0.9798
Average Slope	788.3704	691.5728	689.4852	689.4384

purpose. Despite XMA solubilities being >10 times the maximum expected concentration, 0.09% NaCl solution was chosen as the receptor medium for its physiological relevance, cost-effectiveness, and availability. Among the membranes tested, 0.45 μm PVDF membrane exhibited acceptable recovery, indicating low XMA binding, and was thus selected as the membrane of choice.

Significant differences in XMA release rates were seen in the IVRT release profiles obtained with varying sampling intervals and durations. Over time, release rates decreased and recovery increased, creating a curvature in the release profile that was in close agreement with Higuchi’s square root approximations. The physicochemical characteristics of the formulations may change, leading to a deviation from linearity in the release of API from different semisolid dosage forms. To evaluate the similarity between two gels containing 30% XMA, rather than total release from the gel, Higuchi’s

approximation (recovery $\leq 30\%$) guided the selection of the 5-hour IVRT method with an hourly sampling period. Both positive and negative controls were incorporated to verify the IVRT method's capacity for discrimination, signifying similarity and any discrepancies, instead of assessing total release from the gel. In compliance with the FDA's SUPAC-SS approval requirements, positive and negative controls were added to verify the IVRT method's ability to discriminate, indicating similarities and any differences (Figures 1-7). The IVRT approach proved the similarity between reference and test gels, even with the more stringent acceptance criteria suggested by the latest EMA draft guideline. Studies with positive controls confirmed the IVRT method's capacity to signal similarity while data with negative controls showed that it could identify XMA gel equivalency.

CONCLUSION

The IVRT system's performance verification test (PVT) and apparatus validation met all requirements for assessing API release from topical gel formulations. The IVRT system's consistency and fitness for its intended application are confirmed by the small differences seen during the PVT runs. The validation methods complied with the suggestions indicated in the draft guidelines¹⁶ and FDA guidance.^{3,4} It was then effectively utilized to evaluate "equivalency" in order to introduce a generic XMA gel by contrasting a 30% XMA gel with the Softalia 30% XMA reference. Furthermore, the novel way to illustrate the IVRT method's discriminatory power was to include both positive and negative controls. These controls demonstrated the IVRT method's discriminatory power by satisfying the necessary acceptance criteria for equivalency and non-equivalence, respectively. Based on statistical studies, the suggested IVRT method for figuring out XMA levels in a sample is precise, repeatable, and specific. XMA in semisolid and pharmaceutical dose forms may be detected and evaluated with this HPLC-based IVRT method since the selected mobile phase efficiently separates ximenynic acid.

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