E-ISSN 2250-0944
ISSN 2250-1150
doi: 10.31838/ijprt/13.02.02

Research Article

Formulation and Evaluation of Fravotriptan Transdermal Patches for the Treatment of Migraine

S. VAGDEVI[1], KAMERE KISHORE[2], DR. VIJAY KUMAR GAMPA[3]
[1,2,3]KGR Institute of Technology and Management (Affiliated to Osmania University), Rampally village, Keesara Mandal, Rangareddy, Telangana, India
Received: 14.11.22, Revised: 10.12.22, Accepted: 07.01.23

ABSTRACT
Transdermal patches deliver the drug through the skin in a controlled and predetermined manner in order to increase the therapeutic efficacy of drug. Fravotriptan a 2nd generation triptan that has a favorable tolerability profile and patients have reported greater satisfaction. The aim of the present work was to formulate and evaluate transdermal patches of Fravotriptan. For the current study, the transdermal patch containing drug with different ratios of polymeric combinations and varying plasticizer concentration were prepared. The patch was fabricated by solvent casting method. The casting solvents and plasticizers used were ethanol and propylene glycol respectively. The polymers used were HPMC, PVP and EC. The fabricated patches were evaluated for its physicochemical study. From the studies it concluded that F5 (EC and HPMC of 1:4 ratio and plasticizer 2 ml) whose thickness 0.124 mm, weight variation 110 mg, folding endurance 328 times, percentage moisture absorption of 5%, tensile strength of 0.78 kg/cm^2, drug content of 90.42 % and *in vitro* drug release of 96.3 % may be selected as the optimized formulation. The released kinetics of the optimized formulation follows first order. It can be concluded that it is possible to fabricate a transdermal delivery of Fravotriptan for treatment migraine where the patient acceptance and tolerability profile is high.

KEYWORDS: Fravotriptan, Transdermal Patches, HPMC, Ethyl Cellulose.

INTRODUCTION
Transdermal patches deliver the medicament through the skin in a controlled and predetermined manner in order to increase the therapeutic efficacy of drug and reduced side effect of drug. It delivers the drug via skin portal to systemic circulation at a predetermined rate over a prolonged period with a specific amount of dose. Success of a transdermal patch depends on a variety of biological, physiological, biochemical and biophysical factors. It depends on the composition, integrity and thickness of the stratum corneum. The structure and size of the molecule are indicators of diffusivity. It depends on the permeability of the membrane in the transdermal drug delivery system, state of skin hydration pH and other physiochemical drug properties.
Lipophillicity of the drug, degree of partitioning of the drug and associated components are also essential. Presently this method of drug delivery has the most potential than other routes of administration because it avoids problems like gastric irritation, metabolic variation and due to the presence of food certain food-drug interactions may happen. This delivery system is also useful for unconscious patients. It has certain limitations like slow penetration rates, lack of dosage flexibility and use of low dosage drugs are restricted. Its main advantage is that it avoids first-pass metabolism.[1]
Fravotriptan is a 2nd generation triptan with lots of benefits over other members of its category. Clinical trials have exposed that Fravotriptan is effective to other oral migraine specific agents in the acute migraine treatment and has more consistent long term efficacy across multiple migraine attacks. It is reported that the quality of the life is improved with the use of Fravotriptan. The efficacy and tolerability of Fravotriptan for the acute treatment of migraine have thus been well established.[2]
Migraine is a common disabling disorder, associated with considerable personal and social burden. It affects females approximately 3-22% and males of 1-16%. Results based on US population suggest that migraine affects women approximately 18.2% and men 6.5%. Migraine considered as a burden on the sufferer, friends, family and society in accordance to economic cost and quality of life.[3]

MATERIALS AND METHODS
Fravotriptan benzoate was received as a gift sample from Sms Pharmaceuticals limited Hyderabad. Propylene glycol was from Sisco Research Laboratory, Mumbai. HPMC, PVP, Ethyl

Cellulose from HiMedia laboratories, New Delhi. Ethanol, Disodium hydrogen orthophosphate, Potassium dihydrogen orthophosphate, Sodium hydroxide were from Loba chemie, Mumbai. All the materials were of analytical grades.

Formulation of Transdermal Patch

Preparation of casting solution

Casting solutions were prepared by dissolving weighed quantities of polymers in ethanol under magnetic stirring. Drug and plasticizer were added to this polymer solution and mixed. Volume was made upto 25 ml using ethanol. The entrapped air bubbles were removed by using ultra sonic bath.[4]

Preparation of transdermal patch

A casting solution of 25 ml was poured into the petri dish of surface area 44.5 cm^2 and dried at room temperature for 24 hrs. A films of 2.5 cm2 was cut and stored in desiccator for 48 hrs for further drying. They were then wrapped in aluminium foil and packed in self sealing coversThe formulae for the preparation of transdermal patches of Fravotriptan were given in Table.1

Table 1. Formulation of transdermal patches

Formulation	Drug (mg)	HPMC (mg)	EC (mg)	PVP (mg)	PEG (ml)	Solvent upto (ml)
F1	102	1250	0	200	1	25
F2	102	1250	0	200	2	25
F3	102	1250	0	200	3	25
F4	102	1000	250	200	1	25
F5	102	1000	250	200	2	25
F6	102	1000	250	200	3	25
F7	102	750	500	200	1	25
F8	102	750	500	200	2	25
F9	102	750	500	200	3	25

Characterization of transdermal patch

Physical appearance

Transdermal patches were visually inspected for color, flexibility, homogeneity and smoothness.[5]

Film thickness

The aim of the present study was to check the uniformity of the thickness of the transdermal patches were determined by using screw gauge at 5 different places of the patch.[5]

The mean values were calculated and the results are shown in Table.2

Total reading = *MSR* + *CSR*

CSR = _*mm* × *LC*

Weight variation

Weight variations of the transdermal patches were done by cutting the patches into 1cm2 from 3 different points of the patches and weight of each patch was determined by using the digital balance. The average weight and its standard deviations was calculated.[5] The results were shown in Table.2

Folding endurance

The evaluation of folding endurance of the transdermal patches was done to determine the folding capacity of the film subjected to frequent extreme condition of folding. A strip of specific area 2.5 cm2 was cut evenly and repeatedly folded at the same place till it breaks. The number of times the patches folded at same place without breaking was noted as its folding endurance value. [6] Results are shown in Table 2

Drug content uniformity

The patch of 2.5 cm^2 transferred into a glass stopper flask-containing methanol. The flasks were closed and shaken till the patch was completely dissolved. This solution was filtered and the volume was made upto 100 ml by using buffer of pH 7.4, from this 1 ml of solution was pipette out and was diluted upto 10 ml and the absorbance was measured by UV spectrophotometer at 230 nm.[7] Results are shown in Table 2

Tensile strength

The instrument that was designed in our laboratory was used for the measurement of tensile strength. Mechanical properties of the polymeric patches were conveniently determined by measuring their tensile strength. It consists of a pan hanged by using a strong thread and the other end of the thread was attached with the centre of the patch. The whole instrument was held like a beam balance and weights were gradually added to the pan to increase the pull force till the film was cut. The weights required to break the patch was noted.[7] The tensile strength was calculated using Allen's formula and the results are shown in Table 2

$$\text{Tensile strength} = \frac{\text{Break fource}}{a \times b} \times \frac{1 + \Delta L}{L}$$

Moisture absorption

The prepared transdermal patches were weighed individually and kept in a desiccator for 24 hrs. The patch was then taken out and exposed to atmospheric air for 48 hrs. Average percentage moisture absorption of each film was calculated.[8] The results are shown in Table 2

$$\% \text{ Moisture content} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

In vitro drug release studies

The *in vitro* permeation of the drug from the patches was studied using modified Keshary-Chein diffusion cell. It consists of 2 compartments, the donor compartment and the receptor compartment. The donar compartment was in contact with the ambient conditions of the atmosphere and was in contact with a solution in the receptor compartment, which is pH 7.4 buffer and was stirred by magnetic bead and driven by a magnetic stirrer at temperature 32°C. The samples were withdrawn at the specified time intervals upto 8 hrs and equivalent volume of solution was replaced into receptor compartment after each withdrawal.[9] The results are shown in Table 3

Ex vivo drug release

Modified *ex vivo* permeation apparatus was used for the *ex vivo* drug release studies through pig skin. Phosphate buffer of pH 7.4 was used as the medium. Pig's ear skin was collected from the local slaughter house and cleaned it properly. The collected skin with suitable size was stored at -20 °C. Before the permeation, the pig skin was taken out from the freezer and allowed to cool at room temperature. A patch size of 2.5 cm^2 was cut and placed at the centre of the pig skin. And tied to the specially designed glass cylinder, donor compartment. The glass cylinder was then attached to the metallic shaft and suspended in 50 ml dissolution medium so that the membrane just touched to the receptor surface. Temperature of the dissolution medium maintained at 32°C throughout the experiment and the medium stirred at the 50 rpm using magnetic stirrer. In a specified interval 3 ml sample was taken from the receptor medium and filtered and equivalent volume of the solution must be replaced to the receptor compartment. Samples were diluted and analysed by UV spectrophotometer at 230 nm. All the readings were taken 3 times and average was calculated.[10] The results are shown in Table 4.

Kinetic Studies

Depending upon R and k values obtained from different models, the best- fit model was selected.[11] The kinetic study values given in Table 5

Skin Irritation

The skin irritation study on the healthy male albino rat was conducted after obtained the ethical clearance for handling of experimental animals from the institutional animal ethics committee under the ref: NGSM/IAEC/2016-17/06. The optimized patch chosen and tested for its potential to origin skin irritation in rat. The rat was shaved carefully avoiding the peripheral damage and the patch was applied onto the shaved skin using an adhesive tape. On the previous day of the experiment, the hair on the rear part of rat was removed carefully. The transdermal systems were applied. The applied patch was kept for 7 days and finally the application sites were graded according to a scoring scale.[12] The data on skin irritation is given Table 6.

Stability Studies

Stability of a drug has been defined as the ability of a particular formulation, in a specific container, to remain within its physical, chemical therapeutic and toxicological specification. In a rational design and evaluation of dosage forms for drugs, stability of the active components must be major criteria in determining their acceptance or rejection. For pharmaceutical dosage form, it is necessary to determine the stability test of the dosage form. This will include storage at both normal and exaggerated temperature conditions; with the necessary extrapolations to ensure the product will over its designed shelf life, provided medication for absorption at the same rate as when originally formulated.[13]

ICH tripartite specifies the guidelines for testing the stability of new drugs products, as a technical required for the registration of pharmaceutical for human use. The ICH guidelines have established that long term stability testing should be done at 25 °C at 60 % RH and stress testing should be done at 40 °C / 75 % RH for 6 month. If considerable changes occur at these stress condition, then the formulation should be tested at an intermediate condition (30 °C / 75 % RH). They were then subjected to further evaluation studies and checked for any parameters.[14] .

RESULTS AND DISSCUSSION

Table 2. Effect of prepared patches on physicochemical parameters

Formulation code	Thickness* (mm) Mean ±SD	Weight variation* (mg)	Folding Endurance	% Moisture Absorption*	Tensile strength* (kg\cm²)	% Drug content* Mean ± SD
F1	0.172 ± 0.013	110 ± 0.23	301	0.7 ± 0.023	0.73±0.12	91.8 ± 0.15
F2	0.162 ± 0.015	109 ± 0.31	308	1.0 ± 0.021	0.83±0.15	91.7 ± 0.13
F3	0.136 ± 0.012	108 ± 0.32	288	1.2 ± 0.013	0.93±0.13	91.26 ± 0.16
F4	0.124 ± 0.012	109 ± 0.22	302	0.6 ± 0.032	0.92±0.22	91.88 ± 0.12
F5	0.124 ± 0.013	110 ± 0.32	328	0.9 ± 0.022	0.73±0.17	90.42 ± 0.14
F6	0.142 ± 0.015	109 ± 0.21	350	1.0 ± 0.021	0.76±0.14	91.36 ± 0.12
F7	0.138 ± 0.013	107 ± 0.21	267	0.7 ± 0.014	0.95±0.16	91.22 ± 0.14
F8	0.136 ± 0.016	108 ± 0.32	310	0.8 ± 0.016	0.857±0.21	91.23 ± 0.14
F9	0.139 ± 0.011	109 ± 0.31	281	0.9 ± 0.011	0.91±0.11	90.17 ± 0.12

*Each value is the average of 6 determinations

Table 3. Effect of polymer concentration on *in vitro* drug release profile

Time (min)	Percentage drug released (mg)								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
15	13.8 ± 0.11	16.4 ± 0.11	16.3 ± 0.12	16.9 ± 0.16	14.2 ± 0.13	16.2 ± 0.11	15.4 ± 0.13	16.9 ± 0.16	15.7 ± 0.11
30	19.6 ± 0.16	20.3 ± 0.14	27.5 ± 0.13	23.1 ± 0.11	23.1 ± 0.16	22.1 ± 0.16	21.3 ± 0.16	23.1 ± 0.11	20.8 ± 0.11
60	26.4 ± 0.11	31.1 ± 0.12	36.0 ± 0.13	33.1 ± 0.14	33.5 ± 0.12	33.3 ± 0.16	32.9 ± 0.11	33.1 ± 0.14	33.1 ± 0.13
120	36.2 ± 0.14	40.2 ± 0.11	49.1± 0.12	45.2 ± 0.12	47.4 ± 0.11	45.6 ± 0.11	41.6 ± 0.16	45.2 ± 0.12	42.2 ± 0.12
180	45.6 ± 0.13	51.2 ± 0.12	53.1 ± 0.13	54.7 ± 0.13	56.6 ± 0.13	54.3 ± 0.14	52.6 ± 0.14	54.7 ± 0.13	53.1 ± 0.14
240	53.5 ± 0.13	57.5 ± 0.16	60.1 ± 0.11	62.3 ± 0.14	64.3 ± 0.11	64.3 ± 0.13	57.9 ± 0.16	62.3 ± 0.14	59.3 ± 0.12
300	59.3 ± 0.13	65.5 ± 0.13	71.2 ± 0.16	69.9 ± 0.13	72.1 ± 0.12	73.9 ± 0.16	65.4 ± 0.12	69.9 ± 0.13	66.5 ± 0.14
360	69.0 ± 0.12	75.3 ± 0.11	78.2 ± 0.13	75.8 ± 0.16	80.6 ± 0.14	82.8 ± 0.11	76.3 ± 0.11	75.8 ± 0.16	77.3 ± 0.16
420	76.5 ± 0.13	80.4 ± 0.13	83.4 ± 0.12	82.4 ± 0.11	89.1 ± 0.11	90.7 ± 0.13	82.7 ± 0.16	82.4 ± 0.11	83.8 ± 0.12
480	80.3 ± 0.16	86.6 ± 0.12	87.2 ± 0.13	87.9 ± 0.13	96.3 ± 0.12	96.5 ± 0.13	87.1 ± 0.15	87.9 ± 0.13	90.5 ± 0.14

Table.4. Effect of polymer concentration on ex-vivo drug release

Time (min)	Percentage drug released (mg)								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
15	14.1 ± 0.12	13.6 ± 0.11	14.3 ± 0.14	14.4 ± 0.13	14.2 ± 0.12	12.2 ± 0.11	14.4 ± 0.12	14.3 ± 0.11	14.2 ± 0.14
30	18.6 ± 0.11	17.5 ± 0.12	18.6 ± 0.12	22.3 ± 0.12	20.1 ± 0.13	16.7 ± 0.11	18.3 ± 0.13	18.1 ± o.11	20.1 ± 0.12
60	24.3 ± 0.16	26.7 ± 0.14	24.4 ± 0.13	30.1 ± 0.12	29.6± 0.12	21.2 ± 0.13	22.9 ± 0.12	21.3 ± 0.14	25.3 ± 0.12
120	33.6 ± 0.11	39.3 ± 0.12	36.2 ± 0.14	36.2 ± 0.12	37.4 ± 0.12	35.2 ± 0.13	31.6 ± 0.14	30.3 ± 0.12	35.6 ± 0.12
180	44.2 ± 0.12	43.4 ± 0.14	45.6 ± 0.12	44.4 ± 0.14	46.4 ± 0.12	47.1 ± 0.14	39.6 ± 0.12	39.3 ± 0.12	44.3 ± 0.14
240	53.5 ± 0.11	50.7 ± 0.12	53.5 ± 0.12	52.8 ± 0.11	56.3 ± 0.14	52.1 ± 0.12	47.9 ± 0.14	48.2 ± 0.12	54.3 ± 0.12
300	57.8 ± 0.13	61.2 ± 0.12	62.3 ± 0.15	69.6 ± 0.14	66.1 ± 0.14	68.4 ± 0.14	55.3 ± 0.12	56.5 ± 0.12	61.7 ± 0.14
360	65.3 ± 0.14	72.4 ± 0.13	75.0 ± 0.11	75.8 ± 0.12	77.2 ± 0.14	76.0 ± 0.14	66.3 ± 0.12	66.7 ± 0.14	69.8 ± 0.14
420	69.4 ± 0.11	78.4 ± 0.14	80.5 ± 0.12	82.4 ± 0.12	85.5 ± 0.14	82.4 ± 0.12	72.7 ± 0.12	73.2 ± 0.12	77.7 ± 0.12
480	73.9 ± 0.12	81.6 ± 0.11	84.8 ± 0.14	87.5 ± 0.11	93.2 ± 0.12	93.9 ± 0.14	81.1 ± 0.14	83.5 ± 0.12	84.3± 0.14

In vitro drug release study

The *in vitro* drug release was done as per the procedure given in the methodology by using modified Keshary - Chein diffusion cell.

Kinetic studies

The *in vitro* drug release kinetics based on mechanism of release was evaluated. The regression coefficient R^2 of the drug for different model is shown in the Table 5

Table 5. Kinetic models of formulations

Formulation code	Zero order	First order	Higuchi matrix model	Korsemeyer peppas	
				R^2	N
F1	0.681	0.945	0.894	0.872	0.203
F2	0.768	0.962	0.942	0.879	0.196
F3	0.673	0.979	0.878	0.863	0.207
F4	0.646	0.959	0.870	0.863	0.215
F5	0.645	0.994	0.871	0.863	0.215
F6	0.658	0.992	0.879	0.865	0.212
F7	0.803	0.955	0.960	0.889	0.182
F8	0.827	0.928	0.962	0.894	0.177
F9	0.789	0.928	0.943	0.886	0.183

The release profile of the drug from all the formulations we conclude that it appeared to follow 1st order by considering the regression coefficient.

Skin irritation test

The skin irritation study done on albino rat.. The results noted in table 6.

Table.6. Data on skin irritation

Grade	Sensitivity and reaction
A	No reaction
B	Slight, patchy erythema
C	Moderate, patchy erythema
D	Moderate erythema
E	Severe erythema with or without edema

Stability Studies

Table 7. Effects of appearance and drug content during stability studies stored under 45 °C / 75% RH.

Formulation Code	Days	Appearance	% Drug content
F1	0	Transparent	91.88 ± 0.15
	15	Transparent	91.75 ± 0.13
	30	Transparent	91.66 ± 0.16
	45	Transparent	91.58 ± 0.12
	60	Transparent	91.36 ± 0.11
	90	Transparent	91.21 ± 0.21
F2	0	Transparent	91.70 ± 0.13
	15	Transparent	91. 70± 0.11
	30	Transparent	91.02 ± 0.16
	45	Transparent	90.09 ± 0.12
	60	Transparent	90.05 ± 0.11
	90	Transparent	90.01 ± 0.12
F3	0	Transparent	91.88 ± 0.15
	15	Transparent	91.75 ± 0.13
	30	Transparent	91.66 ± 0.16
	45	Transparent	91.58 ± 0.12
	60	Transparent	91.36 ± 0.11
	90	Transparent	91.21 ± 0.21
F4	0	Transparent	90.42 ± 0.13
	15	Transparent	90.24 ± 0.32
	30	Transparent	90.09 ± 0.12
	45	Transparent	90.05 ± 0.11
	60	Transparent	90.01 ± 0.12
	90	Transparent	89.98 ± 0.32
F5	0	Transparent	91.36 ± 0.22
	15	Transparent	91.21 ± 0.21
	30	Transparent	90.42 ± 0.13
	45	Transparent	90.24 ± 0.32
	60	Transparent	90.05 ± 0.11
	90	Transparent	90.01 ± 0.12
F6	0	Transparent	90.17 ± 0.43
	15	Transparent	90.12 ± 0.13
	30	Transparent	90.10 ± 0.32
	45	Transparent	90.09 ± 0.12
	60	Transparent	90.05 ± 0.11
	90	Transparent	90.01 ± 0.12
F7	0	Transparent	91.32 ± 0.23
	15	Transparent	91.21 ± 0.21
	30	Transparent	91.17 ± 0.43
	45	Transparent	90.42 ± 0.13

	60	Transparent	90.24 ± 0.32
	90	Transparent	90.05 ± 0.11
F8	0	Transparent	90.01 ± 0.12
	15	Transparent	90.17 ± 0.43
	30	Transparent	90.12 ± 0.13
	45	Transparent	90.10 ± 0.32
	60	Transparent	89.98 ± 0.32
	90	Transparent	89.90 ± 0.21
F9	0	Transparent	90.17 ± 0.12
	15	Transparent	90.12 ± 0.13
	30	Transparent	90.10 ± 0.32
	45	Transparent	90.09 ± 0.12
	60	Transparent	90.05 ± 0.11
	90	Transparent	90.01 ± 0.12

CONCLUSION

From the studies performed on the patches by comparing all the parameters F5 whose thickness 0.124 mm, weight variation 110 mg, folding endurance 328 times, percentage moisture absorption of 5%, tensile strength of 0.78 kg/cm2, drug content of 90.42 % and in vitro drug release of 96.3 % may be selected as the optimized formulation. It can be concluded that it is possible to fabricate a transdermal delivery of Fravotriptan for migraine where the patient acceptance and tolerability profile is high. However, the studies are being performed to increase the safety and efficacy of the drugs and also to improve the practical matters such as the experience of the wearer of the patch, and to provide increased duration of action. Other potential improvements include improved transdermal technology that utilizes mechanical energy to increase drug flux across the skin. In recent times, skin considered as a safest port for drug administration, to provide continuous drug release.

REFERENCES

1. Rangasamy M, Prathiban KG. Recent Advance In Novel Drug Delivery System. Int J Res Ayur Pharm. 1(2); 2010: 316-326.
2. Lainez MJ. Fravotriptan in the treatment of migraine. Asian J Neuropsy Disea and Treat. 2(3); 2006: 247–259.
3. Pierce M, Neill C, Felker E, Sebree T. Zelrix: A novel transdermal formulation of sumatriptan. Ame Soci Headache Treat Res. 2(2); 2013: 34-42.
4. Rao NG, Krishna RA. Formulation and *in vitro* drug release studies of oxiconazole nitrate transdermal patches. IJAPBS. 3(1); 2014: 39-42.
5. Keleb E, Sharma RK, Mosa EB. Transdermal drug delivery system- Design and evaluation. Int J Adv Pharm Sci. 1(1); 2010: 201-211.
6. Udupa N. Design and evaluation of captopril transdermal preperations. Int J Pharmaceutics. 154; 1992: 67-77.
7. Aggarwal G. Development, fabrication and evaluation of transdermal drug delivery system- A review. Pharmainfo.net. 7(5); 2009: 39-43.
8. Naseera K, Sajeeth CI. Formulation, optimization and evaluation of matrix type of transdermal system of simvastatin using permeation enhancers. Int J Curr Pharm Res. 4(2); 2012: 79-87.
9. Nainar S, Kingston R. Biopharmaceutical classification system in *in vitro* / *in vivo* correlation: Concept and development strategies in drug delivery. Trop J Pharma Res. 11(2); 2012: 319-329.
10. Nava G, Pinon E, Mendoza L, Quintanar D, Adriana G. Formulation and *in vitro, ex vivo* and *in vivo* evaluation of elastic liposomes for transdermal drug delivery of ketorolac tromethamine. J Pharmaceutics. 3; 2011: 954-970.
11. Dash S, Murthy PN, Nath L. Kinetic modeling on drug release from controlled drug delivery systems. Acta Poloniae Pharma Drug Res. 2(3); 2012 :217-223.
12. Jain S, Goswami M, Bhandari A. Skin irritation study in transdermal patch of chitosan containing trazodone HCl on rat skin. Int J Res Pharm Bio Sci. 2(3); 2011:1082-1084.
13. Bajaj S, Singla D. Stability testing of pharmaceutical products. J Applied Pharma Sci. 2(3); 2012: 129-138
14. WHO-GMP and ICH stability testing guidelines for drug products. The pharmaceutical Sciences-Pharma Pathway. 2.72-2.79.