Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2015, 7(5):26-34
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

An improved validated ultra high pressure liquid chromatography method for separation of Rotigotine impurities in Rotigotine Transdermal Patch

***Avinash S. Patil [1], Shakil S. Sait[2], Girish Deshpande[1], Prakashkumar Acharya[1] and Veeravenkata Srikanth Kaki[1]**

[1]Mylan Laboratories Ltd., Formulation AR&D, Plot No. 34 A & B, ANRICH Industrial Estate Bollaram Jinnaram (Mandal), Medak District, Hyderabad, India
[2]Dr. Reddy's Laboratories Ltd., Generics A R &D, Innovation Plaza, Survey Nos. 42,45,46 & 54, Bachupalli, Qutubllapur, RR dist, AP, India

---

ABSTRACT

A rapid, specific, sensitive ultra high-performance liquid chromatographic (UPLC) method has been developed for determination of Rotigotine impurities and its degradation products in Rotigotine Transdermal Patches. UPLC was performed on a C18 column with "mobile phase A" consist of pH 10.00 buffer solution while "mobile phase B" consist of Acetonitrile. The mobile phase was pumped in a gradient manner at the flow-rate of 0.4 mL min^{-1}. Ultraviolet detection was performed at 225 nm. Rotigotine impurities and degradation products along with process impurities were chromatographed with a total run time of 30 minutes. Calibration showed that response of impurities was a linear function of concentration over the range LOQ to 200% of the target concentration ($r^2 \geq 0.999$) and the method was validated over this range for precision, accuracy, linearity and specificity. For precision study, percentage relative standard deviation of each impurity was <15% (n = 6). The method was found to be precise, accurate, linear and specific. The method was successfully employed for estimation of Rotigotine impurities and its degradation products in finished product formulation.

Keywords: UPLC – Rotigotine, Impurities and Method validation

---

INTRODUCTION

Rotigotine ((S)-(−)-5-hydroxy-2-(N-n-propyl-N-2-thienylethyl)-aminotetralin) is a non-ergolinic dopamine-receptor agonist, which is used for the treatment of the signs and symptoms of early-stage idiopathic Parkinson's disease. Transdermal drug delivery is an attractive alternative compare to oral drug delivery and injections. The chemical structure is shown in Figure 1A [1].

Rotigotine is not official in any pharmacopeias. Based on the literature survey, no official method has yet been developed for their separation and its impurities [7-9].

Several methods have been reported using HPLC with UV and fluorescent detection for the determination of Metoprolol Succinate and Hydrochlorothiazide individually in pharmaceutical dosage forms as well as in biological fluids [1-6]. Thus, application of an UPLC method with high sensitivity and selectivity will find use for the determination of Rotigotine and its degradation products in Transdermal patches.

MATERIALS AND METHODS

2.1 Chemicals and Reagents

Rotigotine and its impurities, Dethienyl Ethyl Rotigotine, Depropyl Rotigotine, Thiophene tosyl Rotigotine , 3-Thiophene Rotigotine, Dithienyl Ethyl Rotigotine, Acetyl Rotigotine,Methyl Rotigotine, Rotigotine Toluene Sulphonic acid ester and Rotigotine Thienyl Ethyl Ether, Acetonitrile (HPLC-grade from J.T. Baker, USA), 70% Aqueous Ethylamine (HPLC-grade from Merck), Hydrochloric Acid, Methanol (HPLC-grade from J.T Baker, USA), Ethanol (HPLC-grade Commercial),Tetrahydrofuran (HPLC-grade from Merck), Hydrogen Peroxide were from Merck (Darmstadt, Germany. Water was purified by a Millipore (Bedford, MA, USA) Milli-Q water-purification system and passed through a 0.22 µm membrane filter (Durapore; Millipore, Dublin, Ireland) before use.

2.2 Equipment

UPLC analysis was performed with a Waters Alliance system equipped with a quaternary solvent manager, sample manager, column-heating compartment, and Photodiode array detector. This system was controlled by Waters Empower software.

The specificity study was conducted by using heating oven, stability chamber and heating mantel (Thermo Lab, India).

2.3 Standard and Sample Preparation

The standard stock solution of Rotigotine Base was prepared by dissolving an accurately weighed amount of working standards in Ethanol, resulting in a concentration of 1.0mg/mL. Above solution further diluted in diluent3 (Mix Tetrahydrofuran and Ethanol in the ratio 20:80 (v/v)) to get a concentration of 5.0 µg/ mL.

The impurity stock solutions for Rotigotine impurities Dethienyl Ethyl Rotigotine, Depropyl Rotigotine, Thiophene tosyl , 3-Thiophene Rotigotine, Dithienyl Ethyl Rotigotine, Acetyl Rotigotine,Methyl Rotigotine, Rotigotine Toluene Sulphonic acid ester, Rotigotine Thienyl Ethyl Ether impurities was prepared by dissolving an accurately weighed amount in diluent, resulting in a concentration of 5.0 µg $mL^{-1}$of each impurity of Rotigotine impurities .

The test solution was prepared by dissolving an accurately weighed portion of the patch, equivalent to 50 mg of Rotigotine in 10mL diluent-1 (Tetrahydrofuran). Rotary shaking at 200rpm for around 30minutes, after that added 40mL of Diluent-2 (Ethanol) and Sonicated for 30minutes with intermittent shaking. Above solution was filtered through 0.45µ Nylon filter Discarded minimum 2ml of initial solution. The clear liquid used for chromatographic analysis.

2.4 Chromatography

The analytes were separated on an Waters UPLC with BEH C18 Column(100mm x 2.6 id) , 1.7µ at column oven temperature of 25^{o}C with a gradient run program at a flow-rate of 0.40 mL min^{-1} (Table 1). Before use, the mobile phase was filtered through a 0.22 µm Millipore filter. UV detection was performed at 225 nm. The sample injection volume was 1.4 µL in partial-loop mode.

2.5 Method Validation

The method was validated for specificity, precision, accuracy, sensitivity and linear range as per the International Conference on Harmonization (ICH) guidelines [9].

2.5.1 Specificity:

A study was conducted to demonstrate the interference from placebo. Sample solutions were prepared by taking the placebo equivalent to the amount present in the sample solution and analyzed as per test method. Chromatograms of placebo preparations are not showing any interference at the retention time of known impurities as well as analyte peaks.

A study was conducted to demonstrate the known impurities interference by spiking the sample solution with all the known impurities at 0.5% spike level and analyzed as per test method. It is found that all the known impurities are separated from each other and also from analyte peaks.

A study was conducted to demonstrate the effective separation of degradants from Rotigotine peak. The drug product was subjected to hydrolysis by refluxing the test solution in 5 N Sodium hydroxide solution at 65ºC for 60min. Similarly the acidic hydrolysis was performed by refluxing test solution in 5N Hydrochloric acid solution at 65ºC for 60min. The neutral hydrolysis was done in water at refluxing temperature of 25ºC in bench top for 2 hours

.Oxidation studies were performed in 30 % Hydrogen Peroxide solution at Bench top for 25min. On photo stability study drug product was sufficiently spread on petri plates (1 mm thick layer) and exposed to sunlight and UV light at ambient conditions for 7 days. Thermal degradation study was performed by heating drug product at 80° C for 24 hours.

Similarly placebo samples were prepared as like as drug product by exposing formulation matrices without drug substance.

Stressed samples were injected into the UPLC system with photo diode array detector by following test method conditions.

2.5.2 Precision:
The precision of test method was evaluated by using six samples spiked with known Impurities at 0.5% level and analyzed as per test method.

2.5.3 ACCURACY:
To confirm the accuracy of the method, recovery studies were carried out by standard addition technique. Samples were prepared in triplicate by spiking all known impurities in test preparation at the level of LOQ , 50%, 100%, 200% of the standard concentration and analyzed as per the test method.

2.5.4 Sensitivity:
Sensitivity of the method was established with respect to Limit of detection and limit of quantification for all known impurities. Series of concentration of drug solution and its impurities were injected, LOD and LOQ established by Signal to Noise ratio method.

Precision was performed at LOQ level for all the known impurities by injecting six replicate injection of each impurity at the concentration obtained from above method.

2.6 Linearity of Detector Response:
A series of solutions of all the known impurities in the concentration ranging from limit of quantification level LOQ to 200% of standard concentration were prepared and injected into the UPLC system.

Application of Developed Method:
The method suitability was verified by analyzing three different strengths of finished product of. The test solution was prepared by dissolving an accurately weighed portion of the patch, equivalent to 50 mg of Rotigotine in 10mL diluent-1. Rotary shaking at 200rpm for around 30minutes, after that added 40mL of Diluent-2 and Sonicated for 30minutes with intermittent shaking. Above solution was filtered through 0.45µ Nylon filter Discarded minimum 2ml of initial solution. The clear liquid used for chromatographic analysis.

RESULTS AND DISCUSSION

Selectivity, sensitivity, resolution, and speed of chromatographic separation were optimized for the UPLC method. The retention times of Dethienyl Ethyl Rotigotine at about 1.88, Depropyl Rotigotine at about 5.54, Thiophene tosyl at 9.41, 3-Thiophene Rotigotine at 12.05, Dithienyl Ethyl Rotigotine at 15.09, Acetyl Rotigotine at 16.22, Methyl Rotigotine at about 18.92, Rotigotine Toluene Sulphonic acid ester at about 20.51, Rotigotine Thienyl Ethyl Ether at about 23.07 and Rotigotine at 12.99 minutes respectively, under the chromatographic conditions described, and the total run time was 30 min. Chromatograms obtained from blank, diluted standard, controlled sample and Test sample spiked with impurities are shown in Figures 2A, 2B, 2C, 2D and 4E respectively.

HPLC system has been proved to be a promising tool for separation with shorter run time. Use of BEH C18 (100x2.6) mm, 1.7µ as stationary phase enabled optimization of UPLC for both peak selectivity and analysis speed. Rotigotine and its impurities were well separated with good peak shape and resolution. No interfering peaks were observed in blank & placebo, indicating that signal suppression or enhancement by the product matrices was negligible.

After satisfactory development of method it was subjected to method validation as per ICH guideline [19]. The method was validated to demonstrate that it is suitable for its intended purpose by standard procedure to evaluate adequate validation characteristics. The result of system suitability parameter was found to be complying acceptable suitability criteria: relative standard deviation of replicate injection is not more than 5.0% The result of specificity study ascertained the known impurities are separated from each other and also from Rotigotine peak and spectral

purity of all exposed samples found spectrally pure [Table 3]. The % RSD of replicate determination was found to be <5 during precision study, which indicates that the method is precise and data of precision study are shown in Table 4. The result obtained in the recovery study were found within the range of 85% to 115% (LOQ to 200%), which indicates that method is accurate and data for the same is given in Table 5 and 6. Sensitivity of method was verified and method is found to be linear, accurate and precise at limit of quantification and data of LOD & LOQ study are given in Table 7 and 8. The calibration curve of all impurities were obtained by plotting the peak area of individual impurity versus concentration over the range of LOQ to 200% and were found to be linear (r = 0.999). The data of regression analysis of the calibration curves are shown in Table 9. The applicability of the method was verified by the determination of impurities in Finished product formulation. The impurity content was found to be satisfactory in the formulation and data shown in Table 9.

Figure 1: Chemical structure of Rotigotine

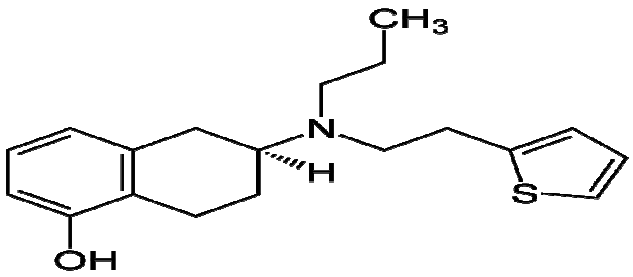


Table-1. Gradient program for elution of Metoprolol and Hydrochlorothiazide and impurities

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–1.44	70 → 70	30 → 30	linear gradient
1.44-8	70 → 50	30 → 50	linear gradient
8-22.60	50 → 15	50 → 85	linear gradient
22.60-26.70	15 → 15	85 → 85	linear gradient
26.70–27.20	15 → 70	85 → 30	Initial gradient
27.2–30.0	70	30	re-equilibration

Table-2. Chromatographic Conditions

LC Column	BEH C18 (100x2.6) mm, 1.7µ
Flow Rate	0.4 mL/minutes
Run Time	30 minutes
Wavelength	225nm
Column oven Temperature	25°C

Table 3. Results of Specificity Study

S.No.	Stress conditions	% Degradation	Individual % Degradation								
			Dethienyl Rotigotine	Depropyl Rotigotine	Thiophene Tosyl Rotigotine	3-Thiophene Rotigotine	Dithienyl Rotigotine	Acetyl Rotigotine	Methyl Rotigotine	Rotigotine Toluene sulfonic Acid	Rotigotine Thienyl Ether
1	Treated with 5N HCl solution 3ml at 65°C temperaturefor about 1hour	2.437	ND	0.085	ND	0.042	0.009	1.249	ND	ND	0.024
2	Treated with 5N NaOH solution 3ml at 65°C temperature for about 1hours	0.399	ND	0.097	0.019	0.05	ND	ND	ND	ND	0.029
3	Treated with 30% H_2O_2 solution 2ml at 25°C temperature for about 25 min	9.197	0.022	0.107	0.103	0.226	ND	0.03	ND	ND	0.023
4	Exposed to Heat at 80°C temperature for about 24 hours	0.527	0.014	0.093	0.018	0.095	ND	0.028	ND	ND	0.026
5	Exposed to Sunlight about 5 hours	0.573	0.017	0.09	0.019	0.093	ND	0.027	ND	ND	0.026
6	Treated with Water 2ml at 25°C temperature for about 2 hours	0.556	0.090	0.017	0.088	ND	ND	0.025	ND	ND	0.026
7	Treated with UV Light	0.697	0.015	0.093	ND	0.153	ND	0.025	ND	ND	0.027

Table 4. Percentage of RSD of impurities in precision study

Nominal concentrations (µg mL^{-1})	Precision (RSD, %) (*n* = 6)
Dethienyl ethyl RTG	0.68
Depropyl RTG	0.89
Thiophene tosyl	1.13
3-Thiophene RTG	0.01
Dithienyl ethyl RTG	0.69
Methyl RTG	1.04
Acetyl RTG	0.88
RTG Toluene SO3 Ester	0.01
RTG Thienyl Ethyl Ether	1.02

Table 5. Percentage Recovery of impurities at different level

Nominal concentrations (µg mL^{-1})	% Mean Recovery of (n = 3)		
	Low@50%	Midle@100%	High@200%
Dethienyl ethyl RTG	105.1	104.3	103.1
Depropyl RTG	108.2	109.1	109.3
Thiophene tosyl	104.2	102.9	101.4
3-Thiophene RTG	103.9	108.8	108.0
Dithienyl ethyl RTG	102.8	109.8	103.3
Methyl RTG	96.3	102.3	100.8
Acetyl RTG	104.0	104.4	102.7
RTG Toluene SO3 Ester	101.9	107.3	105.3
RTG Thienyl Ethyl Ether	103.0	108.3	106.0

Table 6. Percentage of Recovery & precision at LOQ level

Nominal concentrations (µg mL^{-1})	% Recovery	% RSD on precision (n= 6)
Dethienyl ethyl RTG	107.1	9.8
Depropyl RTG	98.4	0.0
Thiophene tosyl	103.3	14.1
3-Thiophene RTG	102.0	0.0
Dithienyl ethyl RTG	100.8	0.0
Methyl RTG	99.7	7.0
Acetyl RTG	103.4	0.0
RTG Toluene SO3 Ester	99.8	0.0
RTG Thienyl Ethyl Ether	97.4	0.0

Table 7. Limit of detection (LOD) and limit of Quantification (LOQ) of impurities in percentage

Nominal concentrations (µg mL^{-1})	LOD in %	LOQ in %
Dethienyl ethyl RTG	0.01	0.04
Depropyl RTG	0.01	0.03
Thiophene tosyl	0.01	0.04
3-Thiophene RTG	0.01	0.03
Dithienyl ethyl RTG	0.01	0.04
Methyl RTG	0.02	0.07
Acetyl RTG	0.01	0.04
RTG Toluene SO3 Ester	0.01	0.04
RTG Thienyl Ethyl Ether	0.01	0.04

Table 8. Correlation Coefficient of impurities

Nominal concentrations (µg mL^{-1})	Slope	Y intercept	Correlation Coefficient
Dethienyl ethyl RTG	5749.9769	-1.0330	0.999
Depropyl RTG	8332.7138	552.0250	0.999
Thiophene tosyl	13987.4315	457.1334	0.999
3-Thiophene RTG	7461.7016	-400.1627	0.999
Dithienyl ethyl RTG	10184.5621	-207.4860	0.999
Methyl RTG	5093.8059	-158.9914	0.999
Acetyl RTG	8255.0976	-199.2792	0.999
RTG Toluene SO3 Ester	9268.8506	16.0735	0.999
RTG Thienyl Ethyl Ether	9768.9511	-7.4631	0.999
Rotigotine	9111.2946	2312.0163	0.999

Table 9. Impurity profile of Finished product formulation

Nominal concentrations (in %)	Finished Product formulations
Dethienyl ethyl RTG	0.017
Depropyl RTG	0.090
Thiophene tosyl	ND
3-Thiophene RTG	ND
Dithienyl ethyl RTG	ND
Methyl RTG	0.027
Acetyl RTG	ND
RTG Toluene SO3 Ester	ND
RTG Thienyl Ethyl Ether	0.028
Maximum Unknown impurity	0.136
% of Total Impurity	0.538

Figure 2A: Typical Chromatogram of Blank

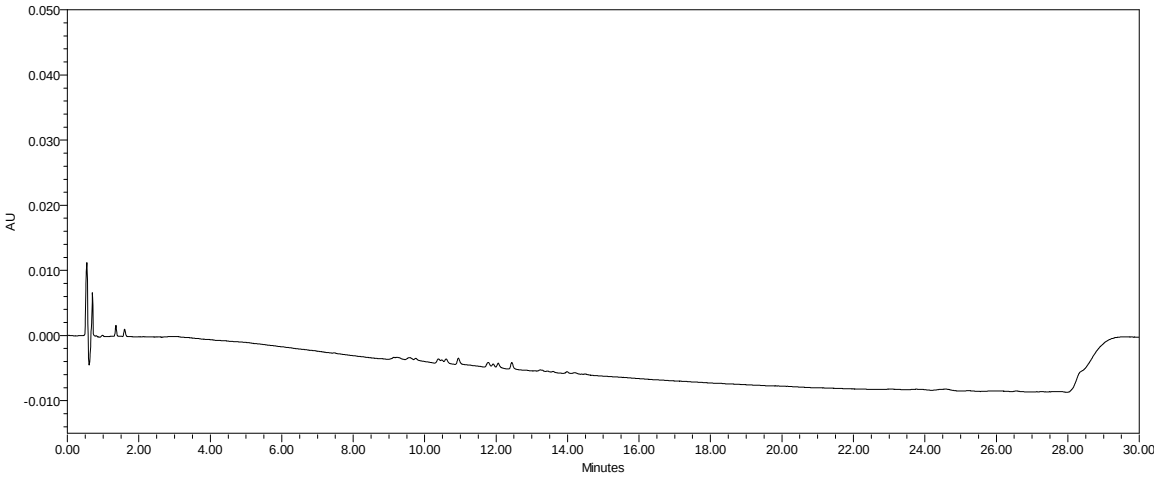


Figure 2B: Typical Chromatogram of Standard Solution

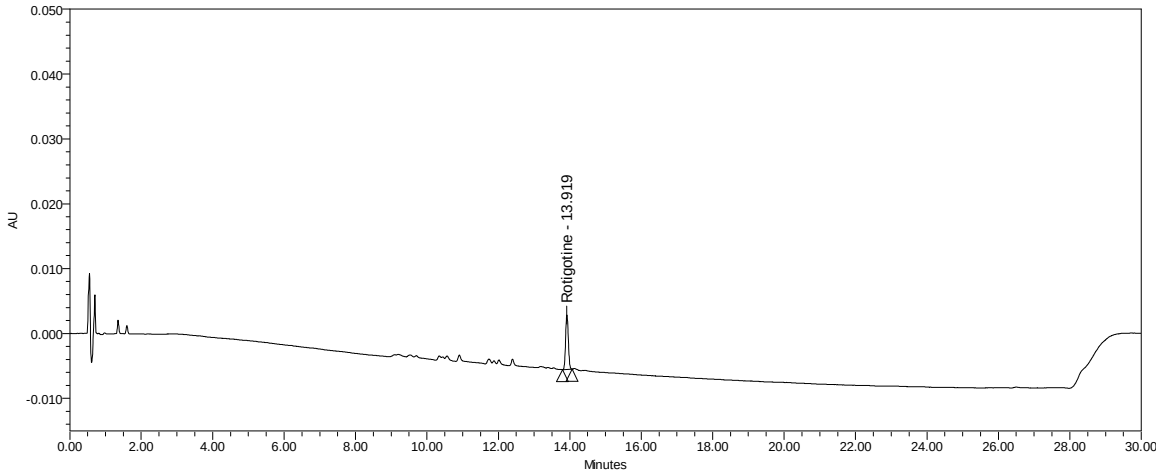


Figure 2C: Typical Chromatogram of Placebo Solution

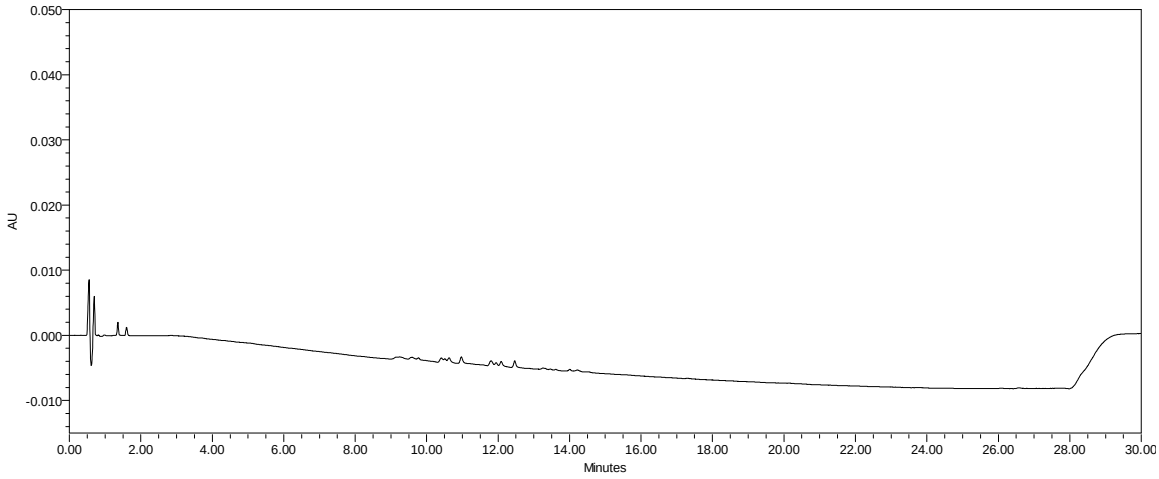


Figure 2D: Typical Chromatogram of Control Sample Solution

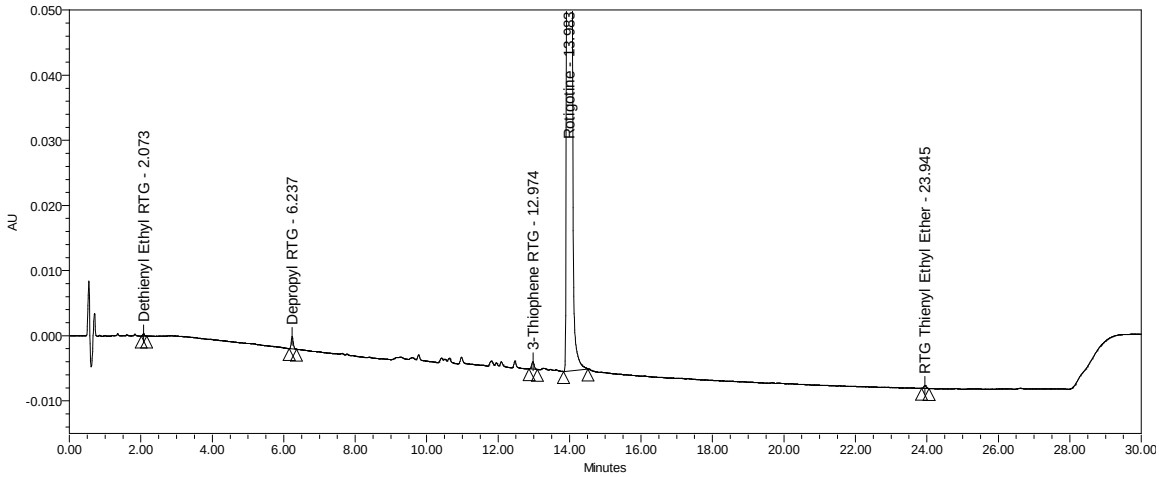
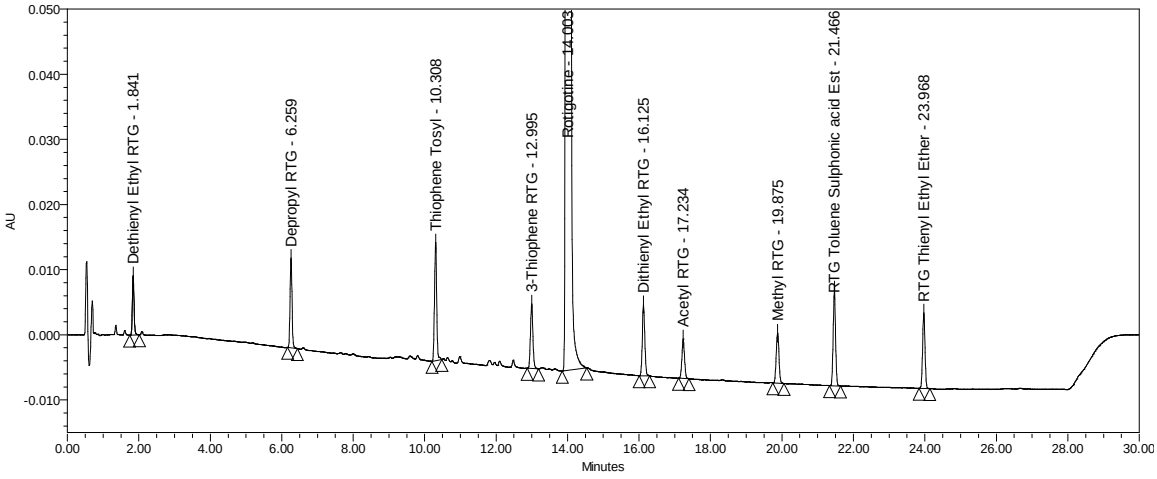


Figure 2E: Typical Chromatogram of Spiked Sample Solution



CONCLUSION

A rapid, specific, sensitive Ultra-performance liquid chromatographic method has been developed for determination of Rotigotine impurities and its degradation products in pharmaceuticals preparation. A number of analytical approaches have been previously described to determine Rotigotine individually in pharmaceutical dosage forms as well as in biological, however, this is the first study reporting a validated reversed phase method for impurity quantification by UPLC in Rotigotine Transdarmal formulation. Based on the literature survey, no official method has yet been developed for their separation and its impurities. The simple and fast UPLC method developed in this study makes it suitable for separation and estimation of impurities without interference from excipients and other related substances present in the pharmaceutical matrices. The analytical performance and the result obtained from analysis of the formulations demonstrated that the method is reliable and sufficiently robust. In conclusion, the high sensitivity, good selectivity, accuracy and reproducibility of the UPLC method developed in this study makes it suitable for quality control analysis of complex pharmaceutical preparation containing Rotigotine and its impurities.

Acknowledgements
We wish to express our sincere thanks to Dr. Abhijit Deshamukh and Mr. Durvas Patil, Mylan Managements for their support and encouragement and Cooperation from colleagues of Mylan Laboratories Ltd.

REFERENCES

[1] Determination of the dopamine agonist rotigotine in microdialysates from the rat brain by microbore column liquid chromatography with electrochemical detection *Journal of Chromatography B*, Volume 845, Issue 1, 1 January **2007**, Pages 109-113.
[2] Reversed-phase liquid chromatographic method with amperometric detection for the determination of the dopamine agonist 2-(N-propyl-N-2-thienylethylamino)-5-hydroxytetralin (N-0437) in human plasma and urine. *Journal of Chromatography B, Biomedical Appl*. 1995 Aug 18;670(2):299-307.
[3] Development and validation of a gradient reversed-phase high-performance liquid chromatographic assay for S(-)-2-(N-propyl-N-2-thienylethylamino)-5-hydroxytetralin (N-0923) from a transdermal delivery system. *Journal of Chromatography B, Biomedical Appl.* **1990** Jun 29;528(2):464-72.
[4] Simultaneous enantioseparation of antiparkinsonian medication Rotigotine and related chiral impurities by capillary zone electrophoresis using dual cyclodextrin system *Journal of Pharmaceutical and Biomedical Analysis*, Volume 46, Issue 5, 14 April **2008**, Pages 854-859
[5] In vivo study on the monoamine neurotransmitters and their metabolites change in the striatum of Parkinsonian rats by liquid chromatography with an acetylene black nanoparticles modified electrode *Journal of Pharmaceutical and Biomedical Analysis*, Volume 72, 18 January **2013**, Pages 74-79.
[6] A Stability indicating of Rotigotine in Bulk Drugs by HPLC Assay method. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, ISSN: 0975-8585.
[7] British Pharmacopoeia. The Stationery Office (onbehalf of Medicines and Healthcare Products Regulatory Agency (MHRA), London, England, **2007**, (vol.1) pp. 1036-1037, (vol. II) pp 1384-1385, (vol.3) pp 2643-2644.
[7] United States Pharmacopoeia and The National Formulary. Asian edition; United States Pharmacopeial convention, INC 12601, Twinbrook Parkway, Rockville, M D, USA,**2003**, pp 909-911, 1220-1222.
[8] Indian Pharmacopoeia. Ministry of Health and Family Welfare, New Delhi, India, **1996**, Vol. 1,pp 371-372.

[9] ICH.Validation of Analytical Procedure: Text Methodology , *U.S Food and drug Administration, IFPMA*: Geneva **2005**