Chapter IV

Method Validation for the Determination of Assay and Stability Indication of Rotigotine by Liquid Chromatography
4.1 INTRODUCTION

The term “stability” of a drug dosage form refers to the ability of a particular formulation, in a specific container, to maintain its physical, chemical, therapeutic and toxicological specifications presented in the monograph on identity, strength, quality and purity. The stability of a drug product should ordinarily be demonstrated by its manufacture by methods appropriate for the purpose [1-4]. Obviously, a stability testing problem is never simple [5]. It is well known that pharmaceutical analysis is always confronted with the tasks of developing analytical methods for stability studies of drugs. Nevertheless, non-specific assay techniques can often result in unreliable measurements of drug stability [6]. A stability-indicating method which can selectively separate each active ingredient from its degradation products, process impurities and formulation excipients, so that the intact drug molecule can be accurately determined which is paramount for stability testing. HPLC was developed during the 1960s and improved during the 1970s. The use of HPLC in the determination of pharmaceuticals, either as a final step of measurement or separation technique, has undergone an enormous expansion in 1980s through technological innovation. Currently, HPLC is widely used in the stability testing of pharmaceuticals. Six special requirements for an HPLC method which may be used for stability testing and therefore be defined as a stability-indicating method can be summarized as follows [7].

1. The peak of the main component (drug substance) should not co-elute with any other peaks originating from its production (by-products). The method should be able to detect the decrease in the active content throughout the period of the stability investigations (stability-indicating assay method).

2. Desired resolutions between the peak of the main component and adjacent peak pairs can be selected to be higher than in other instances to identify possible degradation products similar in structure and chromatographic characteristics, and formed during various storage conditions at low concentrations (stability-indicating purity testing method).

3. The optimum ‘K’ value for the main component is between 5 and 8 to achieve the necessary band spacing for the possible decomposition products with different chemical natures.
(4) The desired value of the precision of stability-indicating assay methods should not exceed ±1.0%, in order that small decreases in active content can be accurately measured.

(5) The peak(s) of decomposition products(s) with different chemical natures should be separated from those of impurities present in the sample at the outset of the investigations, as the results of assay and purity tests are evaluated together and can be corrected using the original impurity content.

(6) Peaks of secondary decomposition products (formed by degradation of by-products and/or decomposition products) can also be separated from other peaks.

Hence, a stability indicating HPLC method should be specific at the time the drug substance is determined by itself, the drug substance content of a dosage form has aged. Also, it should be sufficiently sensitive to detect all low levels of components. Furthermore, a stability-indicating HPLC method is important in the study of drug decompositions which can be the criterion for the product acceptance and must be verified and not assumed. Thousands of papers have been published on the stability of pharmaceuticals. However, the present review focuses exclusively on particles primarily related to the stability-indicating HPLC assay methods of pharmaceuticals. The substances discussed in this review are grouped to assemble those drugs that have similar uses or actions. In addition, the stability-indicating HPLC systems which are conventionally employed for stability testing of pharmaceuticals are tabulated as an aid in selecting an appropriate system for a given in table 4.1 includes the common names of drugs, column types, mobile-phase compositions, the wavelength used and the literature reference. Since 1970, there have been several noteworthy reviews related to the topics of stability-indicating methods. Chafetz [8] extensively reviewed the importance of analytical methodology in drug decomposition. A critical examination of assay methods for representative drugs with respect to their usefulness in establishing and monitoring drug stability was provided and a critical review of some methods for determination of stability was presented. Mollica [9] reviewed the many facts of stability and in addition, included rates, mechanisms and pathways of degradation; dosage forms; marketed product
stability; and regulatory considerations. The review by Taylor and Shivji [10] discussed commonly adopted criteria for a stability-indicating assay and the merits of choosing a reactant or decomposition product for monitoring decomposition. Examples of the application of the initial rate method to simple and complex drug decomposition systems for the determination of decomposition rate constants were also provided in this review. Metha [11] attempted to identify the issues that are critical in the development of an analytical method for the stability testing of drugs in solution. He presented a short review of the analytical techniques used in stability test and emphasized the requirements for stability-indicating analytical method to carry out such tests.

Boehlert [12] discussed a variety of analytical methods used in drug stability studies and presented the need for validation of these methods, emphasizing evaluation of specificity, linearity, precision, accuracy, sensitivity and ruggedness. Illustration of the development of stability-indicating assays is provided, utilizing 5 different drugs representing various types of problems. Kumar and Sunder [13] described various stability-indicating tests, including titrimetry, spectrophotometry, colorimetry and chromatography. Kingsford [14] presented recommendations in designing drug stability trials, in an effort to improve methods currently in use. These include choice of a stability-indicating assay, assay precision, temperature factors relevant to the application results, application of the Arrhenius equation to the drug and the question of whether the accelerated stability trial examines the same degradation reaction observed at room temperature. Carstensen and Rhodes [15] discussed rational policies representative of academia, the pharmaceutical industry, the U.S.Food and Drug Administration, and the U.S.Pharmacopoeia for stability testing, including what parameters should be monitored in the stability testing of pharmaceutical products, stability-indicating assays, retained sample stability testing, fixed date method of stability testing, and stability testing by evaluation of market samples. Dong [16] presented a review, which emphasizes the method development and drug evaluation and with reference to U.S.P. XXII 1990 method validation guidelines and the use of modern LC software instrumentation and diode array detection.
4.1.1 Analgesic and Anti-inflammatory Agents

Hsu of National Laboratories of Foods and Drugs (NLFD) in Taiwan [17] have developed a rapid, sensitive and precise method for the analysis of large numbers of samples containing ketoprofen. Acid, base and photo degradations of the drug were also carried out in this study and different chromatograms were obtained. Carprofen in solid dosage forms and in bulk material, and dichlorophen and a trimer impurity in raw material and veterinary capsules were successfully determined by Ross [18] and Shah [19], respectively. Burce and Boehlert [20] developed a stability-indicating assay for the degradation products of procarbazine HCl using HPLC and reported that the typical range for degradation products in capsules is 0.1-0.5% up to 4.5 years. The determination of chlorpromazine and its degradation products in pharmaceutical dosage forms was presented by Chagonda and Millership [21]. Kubala [22] determined diclofenac sodium in raw materials and pharmaceutical solid dosage forms. Diclofenac sodium and six related compounds (potential impurities and degradation products) can be separated in approximately 43 min. 1-(2,6-dichlorophenyl)-2-indolin-2-one was detected as a degradation product under accelerated aging conditions (20 days at 90°C and 55% r.h.).

HPLC procedures were developed for the determination of some anti-inflammatory drugs incorporated in gel ointments, indomethacin in the controlled-release suppositories, sulindac in tablet formulations, and nefopam hydrochloride and its degradation products by Yamamura [23], Ping [24], Jalal [25] and Tu [26], respectively all these papers studied the stability of the cited drugs and reported that common excipients and/or degradation products yielded no interference. Using HPLC, Kaffenberger [27] determined tebufelone [3, 5-di-t-butyl-4-hydroxyphenyl pent-4-ynyl-ketone] (a new anti-inflammatory drug) strength and studied its stability in bulk drug, dosage formulations and feed a mixtures. Recoveries of tebufelone from bulk drug and capsules and from feed mixtures were greater than 99% and 96 to 102% respectively.

Fabre [28] simultaneously determined oxyphenbutazone and six potential decomposition products by HPLC. The method was more sensitive than TLC and allowed the determination of 0.1% of each degradation product. The technique was applied to the analysis of ointments, tablets and capsules.
4.1.2 Anti-arrhythmic Agents

Das-Gupta [29] developed HPLC procedure for the quantitation of verapamil hydrochloride. The stability of the drug was studied at a range of pH values, and samples decomposed by heating in 0.33M-NaOH for approximately 50 min. were also analysed.

4.1.3 Antibiotics

Hsu and Cheng [30] of NLFD successively quantified cloxacillin in commercial preparations and for stability studies. Thermal degradation products did not interfere with the determination. Latter, Hsu and Fann [31] determined dicloxacillin preparations by another HPLC method which is suitable for potency assays and stability studies. Their results compared well with those of the official microbiological method.

Pavli and Sokolic [32] comparatively determined bacitracin by HPLC and microbiological methods described in B.P. 1980 in some pharmaceuticals and [animal] feed grade preparations. The HPLC yielded more useful analytical information and was particularly useful for rapid and precise determination of bacitracin A, B1 and B2. The microbiological method was found to be less selective. The HPLC indicates that bacitracin B1 is composed of two components and can be used to determined bacitracin F in stability studies.

In connection with the stability and compatibility studies of cephamandole nafate with PVC infusion bags, Faouzi [33] applied an HPLC method developed for the determination of the drug and its hydrolysis product cephamandole in infusion solution of 5%glucose or 0.9% NaCl. No significant drug loss was found during simulated infusions for 1h using PVC infusion bags and administration sets, and no significant differences were found between the two infusion solutions. The stability of the drug (without protection from light) when stored at room temperature for 24h and 4°C for 7 days is discussed. The stability of metronidazole benzoate [benzoyl metronidazole] in liquid preparation and norfloxacin in aqueous solution were investigated by Sa-sa [34] and Nangia [35], respectively, using HPLC. The former drug was found to be most stable at pH 4.
A stability-indicating HPLC method for norfloxacin in tablets using PSDVB [poly (styrenedivinylbenzene)] based stationary phase was developed by Rotar and Lampic [36]. The intra and inter-day coefficients of variation (n=5) were 1.6 and 2% respectively. On the other hand, Chen [37] described the HPLC method for the determination of norfloxacin glutamate and glucuronate in solid and liquid dosage forms. The method was used in thermal stability studies; the shelf-life of the solid and liquid producers was 2 and 1.5 years, respectively. Pan [38] developed a reversed-phase HPLC method for the determination cefoperazone sodium in several common fluids and applied in stability testing, and De-Schutter [39] described a reversed-phase ion-pair high performance liquid chromatographic analysis of tetrazyline [tetrahydrozoline] hydrochloride in nasal preparations. Wang and Yeh of National Defence Medical Center (NDMC) in Taiwan [40] used stability indicating HPLC method to assay cephalzin in pharmaceutical dosage forms.

The HPLC stability-indicating assay for cefuroxime sodium solution in relation to clinical practice was studied by Jackson and Perrett [41]. The HPLC procedure for the measurement of ceftazidime arginine in aqueous solution was developed by Nahata and Morosco [42]. The method was applied to valuation of storage stability of ceftazidime in plastic syringes. Klimes and Zahradnicek [43] selected chromatographic conditions to isolate and identify hydrolytic decomposition products of sulphathiazole. Bergh and Breytenbach [44] analyzed trimethoprim in pharmaceuticals by developed HPLC method. The cited drug and five of its degradation products can be separated using this procedure. Sulphamethoxazole, methyl 4-hydroxybenzoate and propyl 4-hydroxybenzoate, usually present in such formulations, did not interfere. The stability of an aqueous solution of mezlocillin sodium with phosphate buffer solution or other intravenous admixture ingredients such as dextrose, fructose and NaCl determined by HPLC was described by Das-Gupta [45]. The optimum stability was observed at pH 4.8; solution containing dextrose (5%) and NaCl (0.9%) were stable for up to 4 days at 25°C, 36 days at 5°C, and 60 days at -10°C. The stability of sissomicin in hydrophilic petrolatum ointment was investigated by Yamamura [46]. They found little degradation in ointment stored at 5°C in the dark for 9 days. Lauback [47] determined ampicillin in bulk, injectables, capsules and oral suspensions by
reversed-phase ion-pair HPLC. The rapid, precise, stability-indicating assay was specific for ampicillin in the presence of phenylglycine, phenoxyethylpenicillin, 6-aminopenicillanic acid, penicilloic acid and the analogous acid derived from ampicillin. Roy [48] studied the stability of locally manufactured ampicillin trihydrate by HPLC with gradient elution.

4.1.4 Antidiabetic Agents

The quantitation of chlorproparamide and tolbutamide in tablets was developed by Das Gupta [49]. There was no interference from the products of hydrolysis or from excipients in tablets. The products of hydrolysis (4-chlorobenzenesulphonamide and tolune-p-sulphonamide) could be quantified if required.

4.1.5 Antiepileptics

An ion-pair HPLC procedure for the determination of nafimidone [1-(2-naphthoylmethyl) imidazole hydrochloride and its decomposition products were described by Taylor [50]. Use of 85 mm octanesulphonic acid in mobile phase allowed the separation of the acidic decomposition products from the cited drug (but the thermal decomposition product was highly retained). The system with 2 mm tetrabutylammonium bromide, however, was found to be capable of separating all the species.

4.1.6 Antifungal Agents

Christinat and Zulliger [51] developed a stability-indicating HPLC method for the determination of topical preparations of econazole nitrate (Pevaryl) and Pevaryl with triamcinolone acetonide (Previsone) in cream and lotion formulations. Separation of two possible degradation products of Pevaryl from it and the internal standard (miconazole nitrate) was achieved. Bakker [52] described a protocol for quality control and stability testing of cytarabine and its injectable formulations.

4.1.7 Antihistamines

Lo and Krause [53] developed a reversed phase HPLC procedure for the simultaneous determination of probenecid and colchicine in solid dosage form. Tablets were heated at 60°C for 7 days with moisture to study the possible thermal-degradation. The method was found useful for routine quality control and stability
studies. Yuan and Locke [54] investigated the stability of diphenhydramine in liquid and solid drug preparations at room temperature ranging from 42°C to 62°C over a period of 16 weeks using HPLC. It was found that the solid product is more stable than the liquid product and the diphenhydramine is well separated from any interfering peaks.

4.1.8 Antihypertensive Agents

Pharmaceutical dosage forms containing hydralazine hydrochloride and phenyl propanolamine or hydrochlorothiazide (as internal standard) were determined on two columns (30 cm X 3.9 mm) packed with semipolar phenyl or non-polar C18 by Das-Gupta [55]. The method is stability-indicating since the product(s) of decomposition can be separated from the intact drug. The C18 column was recommended for stability studies. Prazosin and polythiazide in the diuretic anti-hypertensive combination product was determined by Bachman [56] using HPLC. The described method can be used for stability-indicating assay of both drug substances and their impurities.

4.1.9 Antimuscarinic Agents

The HPLC was used to study the dissociation constant, partition coefficient, solubility and stability of oxybutynin in vitro by Miyamoto [57]. Oxybutynin was found to be very stable in buffer of pH 1-8, but degraded rapidly at alkaline pH. Degradation half-life was 14 min. at pH 12. Selkirk [58] determined orphenadrine hydrochloride after 99 days storage was 0.06% at room temperature and 1.05% at 50°C. Selkirk [59] determined orphenadrine hydrochloride and its metabolite 2-methylbenzhydrol in dosage forms by reversed-phase HPLC.

4.1.10 Antimycobacterial Agents

Cendrowska [60] analyzed 5-aminosalicylic acid and salicyclic acid in tablets and suppositories using an HPLC method which was used in a stability study. It was found that 1% decomposition of 5-aminosalicylic acid occurred at room temperature over 1 year.
4.1.11 Antineoplastic Agents and Immunosuppressants

Some pharmaceutically important nitro-compounds, furazolidone, nitrofurazone, nitrofurantoin, niridazole and nifuroxime in pharmaceutical preparations were analyzed by Hassan [61]. It was found that exposure to sunlight, the drugs decomposed to 5-nitrofuraldehyde, detected at 311 nm, so the method may be used in stability studies. Fleming and Stewart [62] simultaneously determined cisplatin and 5-fluorouracil in 0.9% sodium chloride for injection. Northcott [63] investigated the determination of ormaplatin by LC. The method was used to establish the stability of ormaplatin in H2O.

4.1.12 Antiviral Agents

Pramar [64] reported a stability-indicating HPLC method for the determination of acyclovir in capsules, ointment and injection formulations. Recovery was quantitative and no interference was observed from excipients. The drug was found to be more stable in alkaline than in acidic solution. Shah [65] determined ribavirin in capsule and syrup related impurities triazole in capsule and syrup. Related impurities triazole carboxylic acid, ribose triazole carboxylic acid and triazole carboxamide were also determined by the same method. A stability-indicating, gradient-elution HPLC assay of Zidovudine in syrup was developed by Radwan [66]. Zidovudine was dissolved (100 mg / 10 mL) in U.S.P.XXI1 syrup and stored in amber glass vials at 50° and 25° for 2 and 17 weeks, respectively, or in colourless glass vials at 25° for 2.5 weeks. Then the HPLC method was applied for the analysis of the drug in commercial preparations.

4.1.13 Anxiolytic Sedative Hypnotics and Neuroleptics

Panaggio and Greene [67] studied the stability of haloperidol using HPLC. It was found that haloperidol samples stored at 25°C were stable for at least 14 days except when exposed to light. Extensive degradation of this drug was noted at temperature above 60°C and at high pH. Beaulieu and Lovering [68] determined perphenazine and its sulphoxide in liquid formulations. It was reported that the calibration graph was rectilinear for 5 to 108 mg of perphenazine sulphoxide and for 0.5 to 1.4 μg of perphenazine. Diazepam and its degradation products were determined by Hewala [69] using HPLC and GLC and applied to a stability study. An HPLC assay for oxazepam tablets and capsules was developed.
by Reif and Deangelis [70]. By this technique oxazepam was separated from all
known degradation products. Degradation products were isolated by TLC and
identified to validate the specificity of the method. Bounine [71] determined
zopiclone in tablets. Zopiclone and its potential degradation products were
separated and simultaneously determined in 15 minutes with detection limits of
0.05-0.2%. Recoveries were 100% with RSD of 0.23-0.48%.

4.2 LITERATURE SURVEY OF ROTIGOTINE

Rotigotine (Neupro) is a non-ergoline dopamine agonist indicated for the
treatment of Parkinson's disease (PD) and restless legs syndrome (RLS) in Europe
and the United States. It is formulated as a once-daily transdermal patch which
provides a slow and constant supply of the drug over the course of 24 hours [72-
73]. Like other dopamine agonists, rotigotine has been shown to possess
antidepressant effects and may be useful in the treatment of depression as well
[74]. Rotigotine was developed by Aderis Pharmaceuticals. In 1998, Aderis
licensed worldwide development and commercialization rights for rotigotine to
the German pharmaceutical company Schwarz Pharma (today a subsidiary of the
Belgian company UCB S.A.) [75]. Neupro, with the active ingredient rotigotine, is
a non-ergolinic dopamine receptor-agonist formulated as a transdermal delivery
system, a patch, designed for once-a-day application. Rotigotine is designed to
mimic the action of dopamine, a naturally-produced neurotransmitter crucial for
proper motor functioning. The system is applied to the skin once a day and
provides rotigotine continuously to the body for 24 hours. Multinational clinical
studies in patients with early stages of Parkinson's disease were completed at the
end of 2003. In 15 clinical trials, more than 1,500 patients with Parkinson's
disease have been treated with rotigotine transdermal system. The clinical trials
have shown efficacy and safety in early Parkinson's disease. Rotigotine exhibits a
low potential of pharmacokinetic drug-drug interactions. The administration of
rotigotine transdermal system offers the convenience of once daily dosing and
easy usage. Rotigotine transdermal system is approved in Europe for the treatment
of patients with early and advanced Parkinson's disease in combination with
levodopa. Since March 2006, the drug has been available on the European market
and has been launched by Schwarz Pharma in 14 countries within Europe, e.g.
Germany, the UK, Austria, Denmark, Ireland, Norway, Switzerland, Sweden,
Greece, Spain, Finland and Poland. Parkinson's disease is a progressive disorder of the central nervous system. The patients – roughly four million worldwide, including approximately one million people in the U.S. suffer primarily from a lack of dopamine, a messenger substance in the central nervous system, which is responsible for the coordination of movement. As a result of this shortage, patients are no longer able to control their movements reliably. Dopamine agonists are drugs that attempt to compensate for this lack of dopamine.

The drug has been approved by the EMEA for use in Europe in 2006 and is today being sold in several European countries. In 2007, the Neupro patch was approved by the Food and Drug Administration (FDA) as the first transdermal treatment of Parkinson's disease in the United States. However, as of 2008, Schwarz Pharma has recalled all Neupro patches in the United States and some in Europe because of problems with the delivery mechanism. General side effects for rotigotine may include constipation, dyskinesia, nausea, vomiting, dizziness, fatigue, insomnia, somnolence, confusion, and hallucinations [76-77]. More serious complications can include psychosis and impulse control disorders like hyper sexuality, punding and pathological gambling [78]. Mild adverse skin reactions at the patch application site may also occur.

The HPLC is a generally accepted method for assay of drug substances. However, recent claims cast doubts on the utility of HPLC assay methods for characterizing quality [S. Gorog, J. Pharm. Biomed. Anal. 36 (2005) 931-937]. This study examines the utility of the traditional drug substance HPLC assay as a quality control parameter. The HPLC assay data from more than 100 batches for each of eight drug substances were compared to results from a mass balance approach (100 - impurities %). Estimates of the variability of the HPLC assays from our data and from the literature ranged from 0.6 to 1.1% R.S.D. This variability is an appreciable portion of a typical acceptance range (98.0-102.0%) and frequently exceeds the variability of the manufacturing process. Therefore, the results of the HPLC assay are questionable at best to determine the acceptability of the drug substance batch. The high variability also can generate a significant percentage of false out-of-specification (OOS) results, even when the "true" purity is 99.0-100.0%. Each false OOS leads to inefficiencies because of unwarranted
investigations for a root cause and/or implementation of countermeasures for a problem that does not exist. Lastly, low precision makes it nearly impossible to detect significant changes in the process mean and/or degradation during a stability study. The use of a mass balance approach for assay retains essentially the same average results as the HPLC assay but gives standard deviations that are up to 10 times less. Monitoring the assay by mass balance allows for more precise process and stability monitoring and facilitates more rapid and accurate identification of process changes.

4.3 AIMS AND OBJECTIVES

The present study has set the following aims and objectives to carry out the research on Method Validation for the Determination of Assay and Stability Indication of Rotigotine by Liquid Chromatography.

1) Development of HPLC method for the Rotigotine.
2) Validation of the Rotigotine and assay method.
3) Forced degradation and solid – state stability indicating nature of Rotigotine.

4.4 EXPERIMENTAL

4.4.1 Materials and Reagents

Reference Standards of Rotigotine obtained from Emmanthi labs, Hyderabad. The HPLC grade Methanol was obtained from Ranbaxy Fine Chemical Limited, New Delhi, India. All other chemical of analytical grade were procured from local sources unless specified. All dilutions were performed in standard volumetric glassware.

4.4.2 Chromatographic Conditions

The instrument used was a Waters Model Alliance 2695 separation module equipped with auto sampler, Waters 2998 PDA Detector and the data recorded using empower software. The mobile phase consisted of buffer and methanol by using the column Zorbax SB C-18, 4.6 X 250mm, and 5μ column and detector of UV at 225 nm, 1.0 mL/min as a flow rate.
4.4.3 Reference solution

Accurately weight and transfer about 5 mg each of Dethienyl ethyl rotigotine impurity, Depropyl Rotigotine impurity and Rotigotine standards and 20 mg of Acetyl rotigotine impurity into a 100 mL volumetric flask, dissolve in and dilute to volume with diluents. Dilute 1.0 mL of this solution to 50 mL with diluents.

4.4.4 Sample solution

Accurately weigh and transfer about 100 mg sample into a 50 mL volumetric flask, dissolve in and dilute to volume with diluents. Prepare in duplicate.

4.4.5 System suitability

Theoretical plates for Rotigotine peak from first chromatogram of standard should be not less than 3000, Tailing factor for Rotigotine peak from first chromatogram of standard not more than 2.0 and % RSD for replicate standard injections not more than 5.0.

4.5 RESULTS AND DISCUSSION

4.5.1 Method Validation

4.5.1.1 Precision

The system precision was performed by analysing system suitability standard solution six times. Results of Peak area of the API and the impurities show that the peak area variation observed for Rotigotine and impurities was less than 5.0%. The results comply with the acceptance criteria and indicating acceptable precision of the system. The percentage relative standard deviation of Peak area of six replicate injections for each impurity is ≤ 5.0.

The Precision of the method was determined by analyzing a sample of Rotigotine solution spiked with impurities at 100% of the specification limit of six replicate sample preparations. The percentage relative standard deviation of recovery obtained for each impurity less than or equal to 5.0. Prior to this, system
suitability parameters were calculated by injecting the system suitability solution. The %RSD was found to be 3.01.

4.5.1.2 Limit of detection and Limit of quantification

The limit of detection (LOD) is determined by calculating the signal to noise ratio and by comparing test results from samples with known concentrations of analyte with those of blank samples and establishing the minimum level at which the analyte can be reliably detected. The result obtained for each impurity is listed in Table 4.1. The limit of detection values obtained for each impurity was within the acceptance criteria. Signal to noise ratio should be about 3:1 and the detection less than 0.15%. Limit of Quantification (LOQ) values were determined from the same experiment as mentioned in the limit of detection section. The LOQ values obtained are in Table 4.2. Signal to noise ratio should be about 10:1 and the quantification limit to be less than level of specification preferably much less.

Table 4.1
Limit of detection (LOD) for Rotigotine and impurities

<table>
<thead>
<tr>
<th>Component</th>
<th>% impurity w.r.to</th>
<th>Concentration (mg/mL)</th>
<th>Signal to noise</th>
<th>LOD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotigotine</td>
<td>0.001</td>
<td>0.00002219</td>
<td>3.5:1</td>
<td>0.001</td>
</tr>
<tr>
<td>Dethienyl ethyl</td>
<td>0.0014</td>
<td>0.00002809</td>
<td>2.7:1</td>
<td>0.001</td>
</tr>
<tr>
<td>Rotigotine</td>
<td>0.0014</td>
<td>0.00002759</td>
<td>3.1:1</td>
<td>0.001</td>
</tr>
<tr>
<td>Acetyl Rotigotine</td>
<td>0.0008</td>
<td>0.00001511</td>
<td>3.5:1</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Table 4.2
Limit of Quantitation for Rotigotine and Impurities

<table>
<thead>
<tr>
<th>Component</th>
<th>% impurity w.r.to Working strength</th>
<th>Signal to noise ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotigotine</td>
<td>0.004</td>
<td>9.7 : 1</td>
</tr>
<tr>
<td>Dethienyl ethyl</td>
<td>0.005</td>
<td>9.5 : 1</td>
</tr>
<tr>
<td>Rotigotine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depropyl</td>
<td>0.005</td>
<td>10.0 : 1</td>
</tr>
<tr>
<td>Rotigotine</td>
<td>0.003</td>
<td>9.9 : 1</td>
</tr>
<tr>
<td>Acetyl Rotigotine</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.5.1.3 Linearity and Range

The linearity of the HPLC method was demonstrated for Rotigotine related substances solutions ranging from LOQ 20%, 40%, 80%, 100%, 120% and 150%. Results obtained are shown in Table 4.3. The linearity results for Rotigotine and impurities in the specified concentration range were found satisfactory, with a correlation coefficient greater than 0.99.

Table 4.3
Linearity of the Rotigotine

<table>
<thead>
<tr>
<th>Component</th>
<th>Slope</th>
<th>Intercept</th>
<th>Correlation coefficient (R)</th>
<th>R²</th>
<th>Intercept value w.r.to 100% Conc. Std.</th>
</tr>
</thead>
<tbody>
<tr>
<td>response</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rotigotine</td>
<td>35373637.75</td>
<td>697.14781</td>
<td>0.9990</td>
<td>0.9979</td>
<td>1.79</td>
</tr>
<tr>
<td>Dethienyl ethyl</td>
<td>29608419.44</td>
<td>-586.1013</td>
<td>0.9999</td>
<td>0.9999</td>
<td>-0.50</td>
</tr>
<tr>
<td>Rotigotine</td>
<td>29608419.44</td>
<td>-586.1013</td>
<td>0.9999</td>
<td>0.9999</td>
<td>-0.50</td>
</tr>
<tr>
<td>Depropyl</td>
<td>30311345.27</td>
<td>375.5343</td>
<td>1.0000</td>
<td>0.9999</td>
<td>1.23</td>
</tr>
<tr>
<td>Rotigotine</td>
<td>66859263.97</td>
<td>-884.5145</td>
<td>0.9996</td>
<td>0.9992</td>
<td>-1.34</td>
</tr>
<tr>
<td>Acetyl Rotigotine</td>
<td></td>
<td></td>
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</tbody>
</table>
4.5.1.4 System Suitability

Prepared the reference solution and the solution were analyzed six times as per the HPLC method described.

4.5.1.5 Accuracy

The accuracy of the method was determined using four solutions containing Rotigotine spiked with the impurities at approximately LOQ, 25%, 50%, 100% and 150% of the working strength of API. % recovery obtained in the range of 100.4 – 100.6 at 80.0% to 120.0%.

4.5.1.6. Robustness

System suitability followed by a sample analysis was run to assess if these changes had a significant effect on the chromatography. A sample of Rotigotine spiked with known impurities was analyzed for verifying the level of impurities at each variation. The retention time of all the impurities including Rotigotine were affected by slight variation in the flow, pH and column temperature, however the system suitability criteria for the method were fulfilled. The number of theoretical plates for Rotigotine peak not less than 3000. The resolution between the peaks is due to intermediate and Rotigotine not less than 2.0. The tailing factor for Rotigotine peak not more than 2.0.

4.5.1.7 Solution stability

A solution of Rotigotine spiked with the impurities and the standard solution stability were kept at room temperature (24-26°C) as well as in the refrigerator at 2-8°C. The solution stability was monitored at different intervals (Initial, 24 hours and 48 hours). No significant variation in the percentage of impurities was observed up to 48 hours at 2-8°C for reference solution and sample solution. The level of unknown impurity was found to increase in the sample solution stored at room temperature. It is recommended to keep the solutions at 2-8°C for analysis. Record the results and assign the stability of the solution based on the experimental data. For a stable solution, the individual impurity values to be within ±0.03 of the original value and the total impurities to be within ±0.10 of the original value.
4.6 DEGRADATION STUDIES ROTIGOTINE

Rotigotine and its impurities were analyzed individually to verify the retention times. In order to assess the stability indicating nature of the HPLC method, Rotigotine samples were stressed by acid, base, hydrogen peroxide, heat and UV radiation. The degraded samples were then analyzed a photodiode-array detector.

Diluent: Water and methanol in the ratio of 30:70 v/v.

4.6.1 Oxidation studies

4.6.1.1 At room temperature

Oxidation of Rotigotine sample was carried out by using four solutions (adding 0.2 mL of 5% hydrogen peroxide solution to 5.0 mL of 1.0 mg/mL stock solution). The peroxide treated samples were kept at room temperature for 3 hours, 6 hours and 24 hours, and then diluted to 100 mL with diluents.

4.6.1.2 At 60°C

Oxidation of Rotigotine sample was carried out by using four solutions (adding 0.2 mL of 5% hydrogen peroxide solution to 5.0 mL of 1.0 mg/mL stock solution). The peroxide treated samples were kept at 60°C for 3 hours, 6 hours and 24 hours, and then diluted to 100 mL with diluents. Observed rapid degradation at 3 hours, hence carried out study for 0 hours, 30 minutes, 1 hour and 2 hour as shown in Table 4.4.
Table 4.4

Assay values for Rotigotine

<table>
<thead>
<tr>
<th>Sample</th>
<th>% Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>At RT for 12 hours</td>
<td>98.3</td>
</tr>
<tr>
<td>At RT for 24 hours</td>
<td>97.3</td>
</tr>
<tr>
<td>Control sample</td>
<td>99.1</td>
</tr>
<tr>
<td>At 60°C for 0 hour</td>
<td>100.5</td>
</tr>
<tr>
<td>At 60°C for ½ hour</td>
<td>100.0</td>
</tr>
<tr>
<td>At 60°C for 1 hour</td>
<td>98.3</td>
</tr>
<tr>
<td>At 60°C for 2 hour</td>
<td>97.0</td>
</tr>
<tr>
<td>Control sample</td>
<td>99.6</td>
</tr>
<tr>
<td>At 60°C for 3 hour</td>
<td>89.6</td>
</tr>
</tbody>
</table>

4.6.2 Heat degradation Stability

Heat degradation of Rotigotine sample was examined by heating 5.0 mL of 1.0 mg/mL stock solution at 60°C for 3 hours, 6 hours and 24 hours, and then diluted to 100 mL with diluent. All these degraded samples were analyzed along with control sample using a photodiode array detector for determining the assay and values shown in Table 4.5.
### Table 4.5

Assay values for heat (60°C) degraded samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>% Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control sample</td>
<td>99.6</td>
</tr>
<tr>
<td>At 60°C for 3 hour</td>
<td>99.7</td>
</tr>
<tr>
<td>At 60°C for 6 hour</td>
<td>99.3</td>
</tr>
<tr>
<td>At 60°C for 12 hour</td>
<td>98.2</td>
</tr>
<tr>
<td>At 60°C for 24 hour</td>
<td>98.2</td>
</tr>
</tbody>
</table>

### 4.6.3 UV degradation

Transferred 5.0 mL of 1.0 mg/mL stock solution into a 100 mL volumetric flask and diluted to volume with diluents exposed the solution to UV light with an integrated near UV energy of not less than 200 watt hours/square meter. All these degraded samples were analyzed along with the control sample using a photodiode array detector for determining the assay and values as shown in the Table 4.6.

### Table 4.6

Assay values for UV light exposed sample solution

<table>
<thead>
<tr>
<th>Sample</th>
<th>% Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control sample</td>
<td>99.6</td>
</tr>
<tr>
<td>After 200 wh/sq mt exposure</td>
<td>100</td>
</tr>
</tbody>
</table>
4.6.4 Solid state stability

Solid state stability was performed by exposing the active pharmaceutical ingredients to white fluorescent light, UV light and heat.

4.6.4.1 Standard preparation

Accurately weighed and transferred about 50.0 mg of Rotigotine standard into a 50 mL volumetric flask, dissolved in and diluted to volume with diluent. Further again diluted the 5.0 mL of this solution to 100 mL with diluents.

4.6.4.2 Exposure to white fluorescent light

About 200 mg of the sample was taken in a petridish and exposed to white fluorescent light with an overall illumination of not less than 1.2 million lux hours. After exposure, transferring 50 mg of sample into a 50 mL volumetric flask, dissolved in and diluted to volume with diluents. Further again diluted the 5.0 mL of this solution to 100 mL with diluents.

4.6.4.3 Exposure to UV light

About 200 mg of the sample was taken in a petridish and exposed to UV light with an overall illumination of not less than 200 wh/sq.mt. After exposure, transferring 50 mg of sample into a 50 mL volumetric flask, dissolved in and diluted to volume with diluents. Further again diluted the 5.0 mL of this solution to 100 mL with diluents.

4.6.4.4 Exposure to heat at 105°C

About 200 mg of the sample was taken in a petridish and exposed to heat at 105°C for 24 hours: observed sample was decomposed, hence exposed at 60°C. Accurately weighed and, transferring 50 mg of sample into a 50 mL volumetric flask, dissolved in and diluted to volume with diluent. Further again diluted the 5.0 mL of this solution to 100 mL with diluents.

4.6.5 Forced degradation of Rotigotine

The retention time and relative retention times (RRT) observed for Rotigotine and its impurities are listed in Table 4.7 and Figure 4.1 shows the
chromatograms of Rotigotine and their impurities (I) rotigotine blank (II) Rotigotine standard (III) Dethienyl ethyl (IV) Depropyl Rotigotine

Table 4.7

Summary of retention time and relative retention time (RRT) values for impurities and Rotigotine

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>Retention Time (Minutes)</th>
<th>RRT values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotigotine</td>
<td>12.50</td>
<td>1.00</td>
</tr>
<tr>
<td>Dethienyl ethyl rotigotine</td>
<td>2.80</td>
<td>0.22</td>
</tr>
<tr>
<td>Depropyl rotigotine</td>
<td>3.56</td>
<td>0.28</td>
</tr>
<tr>
<td>Acetyl rotigotine</td>
<td>25.46</td>
<td>2.04</td>
</tr>
</tbody>
</table>

(I) Rotigotine blank

(II) Rotigotine standard
(III) Dethienyl ethyl

Dethienyl ethyl impurity – 2.800
(IV) Depropyl Rotigotine

Fig. 4.1 Chromatograms of (I) rotigotine blank (II) Rotigotine standard (III) Dethienyl ethyl (IV) Depropyl Rotigotine
REFERENCES


