Method Development and Validation of A Novel Anti-Depressant Bupropion by RP-HPLC

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A B S T R A C T

A speedy, simple and precise RP-HPLC process was developed for the estimation of novel antidepressant drug bupropion with Waters X – Bridge C-18 5µm, 4.6 X 150 mm column using mobile phase Acetonitrile: Ammonium bicarbonate (5mM) pH-9 adjusted with 1% Ammonium hydroxide (%v/v). The flow rate was 1 ml/min and quantification was done by PDA detector at wavelength 254nm. The Bupropion eluted from the column in 5.194 min. The validation was carried out in the light of ICH guidelines with respect to parameters linearity, specificity, accuracy, limit of detection (LOD) and limit of quantification (LOQ). The proposed method showed linearity in the concentration range of 50 to 250 ppm for Bupropion. The linear regression equation of Bupropion was found to be y = 6E+06x + 91344 and correlation coefficient value was found to be 0.997 indicating a high degree of linearity for the drug. The limit of detection (LOD) of bupropion was 0.5 ppm and limit of quantification (LOQ) was 2.0 ppm. The low values of %recovery and %C.V. showed that the method is precise within the acceptance limit of 5% (according to ICH guidelines).

Key words: Bupropion, RP-HPLC, PDA detector, ICH

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INTRODUCTION

Bupropion was first patented in 1974 and released onto the world market in 1985. It was briefly withdrawn due to seizures incidences but reintroduced in 1989 after the daily recommended dose was reduced to lower seizure likelihood. Bupropion is a dopamine and norepinephrine reuptake inhibitor. It is about twice as potent an inhibitor of dopamine reuptake than of norepinephrine reuptake. Besides reuptake inhibition of dopamine and noradrenaline, bupropion also causes the release of dopamine and noradrenaline. Bupropion has numerous therapeutic indications including, depression, smoking cessation, sexual dysfunction, obesity, attention deficit hyperactivity disorder and seasonal affective disorder. It has recently been shown to have anti-inflammatory properties. In 2007 it was the fourth-most prescribed antidepressant in the USA. Bupropion is the water soluble hydrochloride salt of an aminoketone, with a pKa of 7.9. It is also known with the generic name of amfebutamone hydrochloride. Bupropion is a second-generation antidepressant agent that is also used in the management of smoking cessation. CYP2B6 is a polymorphic hepatic enzyme of potential importance in the metabolism of drugs such as Bupropion, efavirenz and cyclophosphamide. Wide interindividual variability in the hepatic expression of CYP2B6 has been reported. In humans, bupropion is extensively metabolized to three principal metabolites (Fig.1.) such as hydroxyl-bupropion or morpholin, erythrohydrobupropion, and theo-hydro-bupropion. The pharmacologically active metabolite hydroxyl-bupropion appears to be the major metabolite, since the plasma levels of hydroxybupropion greatly exceed with respect to those of the parent drug. The cytochrome P450 (CYP) enzyme system, especially CYP2B6, has an important role in bupropion hydroxylation. Also product labeling have indicated that bupropion or hydroxybupropion inhibits CYP2D6. The present study the in vitro hydroxylation of bupropion by the CYP enzyme system was investigated. CYP2B6 was identified to have the major role in hydroxybupropion formation. In addition, we have also investigated the possibility of CYP2D6 inhibition by bupropion or hydroxybupropion.
Analytical Method Validation

Method validation is defined as the process of defining and proving an analytical method acceptable for its intended use. Recent guidelines for methods development and validation for new noncompendial test methods are provided by the FDA draft document, “Analytical Procedures and Methods Validation Chemistry, Manufacturing, and Controls Documentation”. In recent years, a great deal of effort has been devoted to the harmonization of pharmaceutical regulatory requirements in the United States, Europe, and Japan. As part of this initiative, the International Conference on Harmonization (ICH) has issued guidelines for analytical method validation. Method validation is a continuous process. The goal is to ensure confidence in the analytical data throughout product development. Another challenge encountered early in the development of methods intended to support stability studies is ensuring that the method is stability indicating. This process is typically achieved by conducting forced-degradation studies. The design and execution of these studies requires thorough knowledge of the product being tested as well as a good understanding of the analysis technique.

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice. Analytical methods need to be validated or revalidated before introduction into routine use.

- Whenever the conditions change for which the method has been validated (e.g., an instrument with different characteristics or samples with a different matrix); and
- Whenever the method is changed and the change is outside the original scope of the method.
- When quality control indicates an established method is changing with time
- In order to demonstrate the equivalence between two methods (e.g., a new method and a standard).

INSTRUMENTATION

| Instrument       | Description                               |
|------------------|-------------------------------------------|
| HPLC System      | Alliance Waters e2695 Separations Module  |
| HPLC Pump        | Waters 2774 pump                          |
| HPLC Detector    | Waters 2998 Photodiode Array Detector     |
| Software         | Empower Pro                               |
| Column           | Waters X – Bridge C-18 5µm, 4.6 X 150 mm column |
| Injector Loop    | Rheodyne, Model No. 2767, Made in USA     |
| Syringe          | Waters (100 µl)                           |
| Balance          | Electronic Balance Mettler Toledo         |
| Sonicator        | Ultrasonic Aarkey Labtronix Industries PVT Ltd India |
| pH meter         | Thermo Electron Corporation (digital)      |
| Centrifuge machine | Spinwin                                    |
CHEMICALS AND REAGENTS

Working standards of Bupropion were obtained from Analytical Testing Service (unit-II) Okhla New Delhi, India having purity >98%.

| S.No. | Reagents            | Manufacturer          | Grade | Batch No. |
|-------|---------------------|-----------------------|-------|-----------|
| 1.    | Acetonitrile        | Sigma Aldrich         | HPLC  | MMBB2792  |
| 2.    | Ammonium Bicarbonate| Merck Ltd, Mumbai, India | AR   | MK6M552979 |
| 3.    | Ammonium Bicarbonate| Merck Ltd, Mumbai, India | LR   | MA1M610077 |
| 4.    | Methanol            | Sigma Aldrich         | HPLC  | MMBB2881  |
| 5.    | Formic acid         | Sigma Aldrich         | HPLC  | 94318-F   |
| 6.    | Trifluoroacetic     | Merck Ltd, Mumbai, India | HPLC | S6225762  |
| 7.    | Ammonium Hydroxide  | Sigma Aldrich         | HPLC  | 47626512-S |
| 8.    | Sodium Hydroxide    | Finar Reagent         | AR    | 9652333-S3 |
| 9.    | Hydrochloric acid   | Finar Reagent         | AR    | 19085524  |
| 10.   | Hydrogen            | Qualigens Pvt. Ltd.   | AR    | 44273-F   |
| 11.   | Milli Q water       | Millipore (India) Ltd. Bangalore | HPLC |           |

METHOD DEVELOPMENT

Method Development for the Assay of Bupropion:

Chromatographic experiment:

Different chromatographic conditions were tried to optimize the method, which include the following:

Column: - X- BRIDGE C-8 3.5µ, 4.6 X 50 mm
Flow: - 1 ml/min
Detector U.V.: - 214 nm
Injection Volume: - 20µl
Run time: - 10 min
Column temp: - 30°C
Buffer: - 0.5 mm Ammonium acetate
Mobile Phase: - ACN : 0.5 mm Ammonium acetate (50:50 % v/v)

![Figure: 2 Chromatogram of Trial-1](image-url)

| S.No. | RT  | Area  |
|-------|-----|-------|
| 1     | 2.507 | 1743456 |
| 2     | 2.758 | 149821  |
| 3     | 3.073 | 729856  |
Observation - In this condition Bupropion peak not eluted well so change the buffer.

Final Method for Assay of Bupropion

Preparation of Buffer

Accurately weigh and transfer about 450mg ammonium bicarbonate to 1000 ml water, dissolve and adjust the pH to 9.00 ± 0.05 with ammonium hydroxide solution (1%v/v). Filter through 0.45µ or finer porosity membrane filter.

Diluents

Use water: ACN (1:1) as diluent.

Standard stock solution of Bupropion

Accurately weigh and transfer about 10 mg of Bupropion working standard to a 100 ml volumetric flask. Add 50 ml of diluent, sonicate to dissolve and make up the volume with the same upto 100 ml. It become 100 ppm solution.

Optimised Chromatographic Conditions

Table: 2 Optimised Conditions

| S. No. | Parameter            | Optimized Condition                                                                 |
|--------|----------------------|-------------------------------------------------------------------------------------|
| 1      | Instrument (HPLC)    | Alliance Waters e2695 Separations Module                                             |
| 2      | Column               | Waters X – Bridge C-18 5µm, 4.6 X 150 mm column                                      |
| 3      | Mode                 | Gradient                                                                           |
| 4      | Mobile phase         | (ACN: Ammonium bicarbonate buffer (5mM) pH-9 adjusted with Ammonium hydroxide)     |
| 5      | Column Oven temperature | 30°C                                                                                          |
| 6      | Flow rate            | 1 ml/min                                                                            |
| 7      | Detector             | Photodiode array                                                                   |
| 8      | Sample tray temperature | Ambient room temperature                                                                  |
| 9      | Detection wave length | 214nm                                                                                   |
| 10     | Injection volume     | 3µL                                                                                 |
| 11     | Retention time (Rt)  | 5.191                                                                              |
| 12     | Run time             | 10 min                                                                              |

Table: 3 Flow Gradient used during development

| TIME (min) | FLOW ml/min | BICARBONATE | ACETONITRILE |
|------------|-------------|-------------|--------------|
| 0.00       | 1.00        | 50%         | 50%          |
| 1.00       | 1.00        | 50%         | 50%          |
| 6.00       | 1.00        | 10%         | 90%          |
| 8.5        | 1.00        | 10%         | 90%          |
| 9.00       | 1.00        | 50%         | 50%          |
| 10.00      | 1.00        | 50%         | 50%          |

Figure: 3 Chromatogram shows the response of Bupropion after analytical method development.
Purity Data

![Purity plot of Bupropion after development](image)

**Table 4: Purity data**

| Name   | Retention Time (Min) | Purity Angle | Purity Threshold | % Area | Height     |
|--------|----------------------|--------------|------------------|--------|------------|
| Bupropion | 5.191              | 0.257        | 1.025            | 100%   | 700956 µV |

**METHOD VALIDATION**

**Specificity**

Specificity of the method was done by comparing the chromatogram of drug with the chromatogram of blank (mobile phase). The chromatograms of blank, and drugs are given below:

![Chromatogram of Blank (Mobile Phase)](image)

![Chromatogram of Bupropion](image)
LINEARITY

Six different concentration of the drug were prepared for linearity studies. Response was measured as peak area. The calibration curve was obtained by plotting peak area (mv.s) against concentration (µg/ml). The standard curves of all three drugs in the mixture obtained from analysis at 1st, 3rd and 8th days are given below:

![Chromatogram of Bupropion](image1)

![Chromatogram of 50 ppm concentration of Bupropion](image2)

![Chromatogram of 100 ppm concentration of Bupropion](image3)
Figure: 10 Chromatogram of 150 ppm concentration of Bupropion

Figure: 11 Chromatogram of 200 ppm concentration of Bupropion

Figure: 12 Chromatogram of 250 ppm concentration of Bupropion
Analysis of Quality Control Solutions

Quality control solutions for the Bupropion in the concentration range of low (50 ppm), medium (100 ppm), high (250 ppm) was prepared and three injections of each concentration was made quality control. The purpose of quality control solutions was to check the performance of the instrument before analysis of test solution, or to confirm whether the instrument gave constant results or not, by comparing the data of standard solutions with that of quality control solutions.

Chromatogram of low concentration (50 ppm solution) of Bupropion

![Chromatogram of low concentration (50 ppm solution) of Bupropion](image)

Table: 5 Linearity data

| CONC IN PPM | AREA   | R²     |
|-------------|--------|--------|
| 50.0        | 6326293| 0.997  |
| 100.0       | 11560200|       |
| 150.0       | 17972287|       |
| 200.0       | 23431070|       |
| 250.0       | 27947593|       |

Figure: 13 Linearity graph b/w absorbance & concentration

Figure: 14 Chromatogram of 1 injection of 50 ppm solution
Figure: 15 Chromatogram of II injection of 50 ppm solution

Figure: 16 Chromatogram of III injection of 50 ppm solution

Figure: 17 Chromatogram of I injection of 150 ppm solution
Figure: 18 Chromatogram of II injection of 150 ppm solution

Figure: 19 Chromatogram of III injection of 150 ppm solution

Chromatogram of low concentration (200 ppm solution) of Bupropion

Figure: 20 Chromatogram of I injection of 200 ppm solution
RESULT AND DISCUSSION

This study describes a highly sensitive, accurate and reproducible HPLC method for the determination of Bupropion because no such method was developed.

Instrumentation

A method has been developed by experimentation based on the literature survey and ascertained by statistical parameter of sampling using a Alliance Waters e2695 Separations Module from USA which is equipped with a Waters 2774 pump, Waters 2998 Photodiode Array Detector and a injector loop made of Rheodyne, Model No. 2767, Made in USA having a injection volume of 20 µl.

Drug’s identification and characterization

Identification and purity of drug obtained by using UV, IR, NMR spectroscopy, mass spectroscopy and melting range determination.

Bupropion

$^1$H NMR: δ

IR:

ESI-MS: 240 (M+1).

Melting range: 270-272 °C

$\lambda_{\text{max}}$: 242.6 nm.

Method Development

Drugs Solubility

Solubility in different organic and aqueous solvents determined the best composition of the sample solvent. Bupropion was freely soluble in methanol, soluble in ACN and in mobile phase and slightly soluble in water.

Mobile Phase Selection

Different mobile phase were tested but adequate separation of drugs was found in acetonitrile: 5mM Ammonium bicarbonate buffer (50:50 %v/v).

pH Selection

By altering the pH of mobile phase separation of peak was observed. The pH of mobile phase was adjusted to 7.0, 8.0, 9.0 and 10.0. At pH 9.0 satisfactory separation of the drug with good resolution and short run time was achieved. At pH 7.0, 8.0 and 10.0 low retention time of Bupropion and poor separation. So mobile phase acetonitrile: 5 mM
Ammonium bicarbonate buffer (50:50% v/v) pH 9.0 was selected for method development.

**Wavelength Selection**

Maximum absorbance of Bupropion at 242.6nm was determined in mobile phase by utilizing Waters 2998 Photodiode Array Detector. Maximum peak height of drug was obtained at 242 nm by injecting the 3µg/ml concentration of sample of the drug and allows to run at different wavelength.

**Flow rate programming**

For the purpose of rapid analysis of the drug flow rate programming was used which result in shorter run time. Best results were obtained with flow rate programming of selected mobile phase. Mobile phase was started at a flow rate of 1.0 ml/min which was continued for 1.0 min to 10.00 min.

**Optimized Chromatographic Conditions**

As a result of several above experiment steps optimized conditions were selected and a simple, rapid and sensitive method was developed.

| S. No. | Parameter                      | Optimized Condition                                                                 |
|--------|--------------------------------|--------------------------------------------------------------------------------------|
| 1      | Instrument (HPLC)              | Alliance Waters e2695 Separations Module                                             |
| 2      | Column                         | Waters X – Bridge C-18 5µm, 4.6 X 150 mm column                                       |
| 3      | Mode                           | Gradient                                                                            |
| 4      | Mobile phase                   | (ACN: Ammonium bicarbonate buffer (5mM) pH 9 adjusted with Ammonium hydroxide)       |
| 5      | Column Oven temperature        | 30°C                                                                                 |
| 6      | Flow rate                      | 1 ml/min                                                                             |
| 7      | Detector                       | Photodiode array                                                                    |
| 8      | Sample tray temperature        | Ambient room temperature                                                             |
| 9      | Detection wave length          | 214nm                                                                                |
| 10     | Injection volume               | 3µL                                                                                  |
| 11     | Retention time (Rt)            | 5.191                                                                                |
| 12     | Run time                       | 10 min                                                                               |

**Method Validation**

Validation of the developed and optimized HPLC method was carried out with respect to the parameters such as specificity, linearity, stability, accuracy, precision and limit of quantification (LOQ), limit of detection (LOD) in the light of internationally accepted ICH guidelines.

**Specificity**

The specificity of the method was determined by comparing the chromatograms obtained from the sample containing Bupropion standard stock with those obtained from test sample of Bupropion and blank of that. The specificity study revealed at the absence of interference of impurities with the drug since no extra peak appeared at the Rt of drug.

**Linearity**

For linearity studies, even different concentrations of the drug were prepared. The response was measured as peak area. The calibration plot was generated by replicate analysis at five concentration levels and the linear regression equation were calculated using the least square method within Microsoft Excel® program. The calibration curve obtained by plotting the peak area (y) versus analyte concentration (x) in µg/ml showed linearity in the concentration range of50 to 250 ppm. The linear regression equation is y = ax ± b, where a, is slope of the curve and b is the intercept. On the basis of following result typical linear regression equation was found to be y = 6E+06x + 91344. A correlation coefficient value was found to be 0.997 for the drug indicates a high degree of linearity.

**Accuracy**

The accuracy of method was determined by calculation of % recovery. Recovery is typically determined by comparing the response of the method to a reference material with the known value assigned to the material. If the recovery of the assay was poor (e.g. less than 90%) it would be a good indication that there is a problem with the method. The developed method for Bupropion is a valid method.

**Precision**

The precision of method was established by carrying out analysis of the analyte based on standard deviation or relative standard deviation of result from six injections at two different concentrations of drug and working standard solutions. If % RSD of the assay is > 2% then the developed method is not a presided method.

**Stability**

The stability of the analyte solution was determined by treating the analyte in different conditions (alkali, acid, peroxide & elevated temp) at interval of 1st day, 2nd day, 3rd day, 4th day and 5th day. The stability of solution was determined by comparing the response of the method to a reference solution of the drug Bupropion was not degraded (stable) under acid and peroxide condition and also in elevated temperature (60°C).
upto 5 days study but it was degraded in basic condition in just 3 day.

**Limit of Detection (LOD) and Limit of Quantification (LOQ)**

| Drugs     | LOD (ppm) | LOQ (ppm) |
|-----------|-----------|-----------|
| Bupropion | 0.5       | 2.00      |

**Robustness**

Robustness is the degree of reproducibility of results obtained by the analysis of the same sample under a variety of normal test conditions i.e. different analysts, laboratories, instruments, reagents, assay temperatures, small variations in mobile phase, different days etc. (i.e. from laboratory to laboratory, from analyst to analyst.)

| S. No. | Temp | Flow       | R.T. (min) | Area     |
|--------|------|------------|------------|----------|
| 1      | 25°C | 0.9 ml/min | 5.526      | 6985492  |
| 2      |      | 1.0 ml/min | 5.346      | 3938026  |
| 3      |      | 1.1 ml/min | 4.817      | 11994681 |
| 4      | 30°C | 0.9 ml/min | 5.545      | 9668377  |
| 5      |      | 1.0 ml/min | 5.199      | 12316977 |
| 6      |      | 1.1 ml/min | 4.803      | 12508314 |
| 7      | 35°C | 0.9 ml/min | 5.611      | 5457399  |
| 8      |      | 1.0 ml/min | 5.187      | 10490406 |
| 9      |      | 1.1 ml/min | 4.803      | 12373100 |

After changing the temperature and flow rate it was observed that as flow rate increases from 0.9 ml/min to 1.1 ml/min retention time decreases and area increases in an acceptable limit of 0.8 ml to 1.2 ml.

**CONCLUSION**

Developed assay method is simple, rapid, accurate, precise, economical, specific and reproducible for the qualitative and quantitative determination of Bupropion with good resolution in short time and high sensitivity.

In the present work the analyte was separated in a short run. Optimization of the method showed that apart from the mobile phase pH and composition, flow rate is an important crucial parameter.

The selection of gradient mobile phase and flow rate, cut down over all time of sample analysis and thereby made the method most cost effective and rapid. Wavelength selection made the method more sensitive.

It was concluded that the developed method offers several advantages such as rapidity, simple mobile phase and sample preparation step, improved sensitivity makes it specific and reliable for its intended use. This method can be applied to analysis of pharmaceutical dosage forms.

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**REFERENCES**

1. Mehta NB. Meta chloro substituted-alpha butylaminopropiophenone, United States Patent, 1974; 3:819,796.
2. Stahl SM, Pradko JF, Haight BR, Modell JG, Rockett CB, Learned-Coughlin S. A review of the neuropharmacology of bupropion, a dual norepinephrine and dopamine reuptake inhibitor. Primary Care Companion to the Journal of Clinical Psychiatry. 2004; 6(4): 159- 166.
3. Hari Shankar Sharma. International Review of Neurobiology, New Concepts of Psychostimulant Induced Neurotoxicity In Hugo R. Arias, Abel Santamaria, Syed F. Ali, editors. Chapter 9 – Pharmacological and Neurotoxicological Actions Mediated By Bupropion and Diethylpropion. 2009; 88:223-255.
4. Zung WW, Brodie HK, Fabre L, McLendon D, Garver D. Comparative efficacy and safety of bupropion and placebo in the treatment of depression. Psychopharmacology(Berl). 1983; 79(4):343-347.
5. Lief HI. Bupropion treatment of depression to assist smoking cessation. American Journal of Psychiatry, 1996; 153:442a-442.
6. Labbate LA, Grimes JB, Hines A, Pollack MH. Bupropion treatment of serotonin reuptake antidepressant-associated sexual dysfunction. Annals of Clinical Psychiatry, 1997; 9(4):241-245.
7. Plokdowski RA, Nguyen Q, Sundaram U, Nguyen L, Chau DL, St Jeor S. Bupropion and naltrexone: a review of their use individually and in combination for the treatment of obesity. Expert Opin Pharmacotherapy. 2009; 10(6):1069-1081.
8. Cantwell DP, ADHD through the life span: the role of bupropion in treatment. Journal of Clinical Psychiatry. 1998; 59 (4): 92-94.
9. Modell JG, Roisenthal NE, Harriett AE, A.Krishen, Asgharian A, Foster VJ, Metz A, Rockett CB, Wightman DS. Seasonal effective disorder and its prevention by anticipatory treatment with bupropion XL. Biological Psychiatry. 2005; 58(1):658-667.
10. Brustolim D, Riberio-dos-Santos R, Kast RE, Altschuler EL, Soares MB. A new chapter opens in anti-inflammatory treatments: the antidepressant bupropion lowers production of tumor necrosis factor-alpha and interleukin-1 in mouse. International Immunopharmacology, 2006; 6(6):903-907.
11. Schroeder DH. Metabolism and kinetics of Bupropion. Journal of Clinical Psychiatry. 1983; 44(5 Pt 2): 79 – 81.
12. Bryant SG, Guernsey BG, Ingrimb NR.Review of bupropion. Clinical Pharmacology, 1983; 2(6):525-537.
13. Johnston AJ, Ascher J, Leadbetter R, Schmitz VD, Patel DK, Durcan M, Bentley B. Pharmacokinetic optimization of sustained release bupropion for smoking cessation.Drugs. 2002; 62:11-24.
14. Jinno H, Tanaka-Kagawa T, Ohno A, Makino Y, Matsushima E, Hanioka N, Ando M. Functional characterization of cytochrome P450 2B6 allelic variants. Drug Metabolism and Disposition: 2000; 28(10):1176-1183.
15. Hesse LM, Venkatakrishnan K, Court MH, von Molte LL, Duan SX, Shader RI, Greenblatt DJ. CYP2B6 Mediates the In Vitro Hydroxylation of Bupropion: Potential Drug Interactions with Other Antidepressants. Drug Metabolism & Disposition: the biological fate of chemicals, 2003; 31(4):398-403.
16. Hesse LM, Venkatakrishnan K, Court MH, von Molte LL, Duan SX, Shader RI, Greenblatt DJ. CYP2B6 Mediates the In Vitro Hydroxylation of Bupropion: Potential Drug Interactions with Other Antidepressants. Drug Metabolism & Disposition. 2000; 28(10):1176-1183.
17. Biswas, K.M.; Castle, B.C.; Olsen, B.A.; Risley, D.S.; Skibic, M.J.; Wright, P.B. A simple and efficient approach to reversed-phase HPLC method screening. Journal of Pharmaceutical and Biomedical Analysis. 2009; 49:692-701.
18. Staut, T. H.; Dorsay, J. G. High Performance Liquid Chromatography. In: Ohnnesian, L.; Streeter, A. J. Handbook of Pharmaceutical Analysis. 1st ed.; Marcel Dekker, Inc. New York, 2005; 117:87-90.