IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 11, Issue 5 Ver. III (Sep. - Oct.2016), PP 24-29
www.iosrjournals.org

Simultaneous determination and estimation of Amlodipine andPerindopril in raw and tablet formulation by stability-indicating RP-HPLC method

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Abstract: *A sensitive, feasible RP-HPLC method has developed and validated for the analysis of Amlodipine with Perindopril argininein raw and tablet formulation.Successful separation of drugs products is developed on a C(18) column reversed-phase using and using mobile phase composition of Methanol:Phosphate buffer(27:73 v/v). The flow rate was adjusted to 1.1 mL/minute and the absorption maxima were observed at 270 nm utilizing Shimadzu SPD-20A Prominence UV-Vis detector.Good linearity was obtained in the range of 2-10 µg/ ml, 3-15 µg/ml, for Amlodipine,Perindopril arginine respectively. TheHPLC, tabletformulation assay shows percentage purity ranging from 99.16 to 100.18% for Amlodipine, 99.58 to 100.23% for Perindopril arginine. The mean percentage purity is 100.01% and 100.08% for Amlodipine andPerindopril arginine respectively. The chromatographic retention time ofAmlodipine andPerindopril arginine was found to be 4.2 and 7.3 minutes respectively. The tailing factor was 0.769 and 0.780 for Amlodipine and Perindopril arginine respectively. The developed method validated according to the ICH guidelines. The method was found to be applicable for determination and validation of Amlodipine andPerindopril arginine in combined tablet form.*

Keywords: *Amlodipine (AML), Perindopril arginine (PEA), HPLC and UV.*

I. Introduction

Multiple therapies are becoming extremely useful in pharmaceutical dosage forms. As the result, numerous and various combinations of drugs are being introduced into the market. Out of these, anti-Hypertensive drugs are one of the mostly prescribed cardiovascular drugs. Amlodipine is a drug used to lower blood pressure and prevent the pain in the chest. It belongs to a group of drugs known as calcium channel blockers [1,2].Amlodipine inhibits calcium ion influx across cell membrane. The chemical name of Amlodipine is [2-(Amino ethoxy) methyl] - 4 - (2-chlorophenyl) - 3 - ethoxycarbonyl- 5- methoxycarbonyl-6-methyl 1,4dihydropyridine benzene Sulfonate [2].Perindopril, or perindopril arginine, is a long-acting ACE inhibitor [3-4]. ACE (angiotensin-converting enzyme) inhibitors, including peridopril, are commonly used to treat high blood pressure, hypertension, heart failure, or stable coronary artery disease [5]. Perindopril Argininеis chemically 2*S*,3*aS*,7*aS*)-1-[(2*S*)-2-{[(2*S*)-1-ethoxy-1-oxopentan-2-yl]amino}propanoyl]-octahydro-1*H*-indole-2-carboxylic acid [6].

The main function of this ACE inhibitor is to decrease the action. The molecular formula of perindopril arginine and perindopril erbumine is different but their therapeutic action is very similar[6]. Literature review shows several methods has been developed and reported for AML andPEAestimation in biological fluids and there are some methods reported by [10], spectroscopy [11-13], HPTLC, HPLC, UPLC and capillary electrophoresis [14-16]. Two methods were reported for estimation of this combination first is UV spectroscopy [17-19] and the other is HPTLC method [20,21]. Method development of HPLC estimation for this combination is new method will fulfil all requirements of validation according to ICH guidelines.

II. Materials And Methods

The working standard of Amlodipine,Perindopril argininewas purchased from Sigma, UK. The Marketed sample ofPrestaliastrength Amlodipine2.5mg Perindopril arginine3.5mg manufactured by PatheonPharamcceuticals and marketed by Symplmed Pharmaceuticals USA purchased from the local Pharmacy, Germany. Methanol HPLC grade was purchased from Merck, Darmstadt, Germanyand phosphoric acids purchased from Fisher Scientific (UK).

III. Instrumentation

3.1. HPLC instrumentation and chromatographic condition:

HPLC system of Shimadzu LC-20 AT, with an auto sampler (SIL-20ACHT, Shimadzu, Japan) and SPD-10 detector (SPD- M20A, Japan) was used. For data recording the LC-solution software used. A Zorbax Eclipse Plus, Agilent Technology column (150mm x 4.6mm, 5µm) was used Pore size of the column 95Å. For

degassing mobile phase, power sonic 505 ultrasonic baths (Hwashin technology, Seoul, Korea) was used. By using oven (CTO-20AC) column was maintained at a temperature of 39ºC and 1.1 ml/min was the flow rate. Analysis was carried over with 20µl injection volume using SPD-10 detection at 270nm. 15 minutes was set as run time.

3.2. Preparation of Mobile phase: Phosphate buffer was prepared using1.35gof KH_2PO_4 in 500 ml of HPLC grade water by using phosphoric acid pH adjusted to 6. It was filtered with 0.45µ membrane filters and degassed in an ultrasonic bath for 10 minutes. The ratio of final mobile phase isMethanol: phosphate buffer (27:73 v/v).

3.3. Preparation of Amlodipine (AML),Perindopril (PEA) Stock solution: Accurately 1 mg of AML (RS) andPEA (RS) was taken separately in 100 ml volumetric flasks and mixed with 25 ml of mobile phase solutionand sonicated for 10 minutes and 75ml of mobile phase was added to the mark and cooled to room temperature. To get the concentration of 2-10µg/mlof AML and 3-15µg/ml of PEAvarying quantities of standard stock solution was diluted with mobile phase. The twoAML and PEApowder freely soluble in methanol and does not have any interference in the absorption peaks.

3.4. Preparation of sample solution: 10 tablets of marketed sample of Prestalia weighedaccurately andpowder equivalent of 2.5 mg of AML and 3.5 mg PEA transferred into 25mlvolumetric flasks and dissolved with 25 ml mobile phase and the resulting solution was filtered through Whatmanfilter paper.Further dilutions were made based on the required concentrations.

3.5. Method validation: The present method was preceded to obtain new, sensitive and easy method for simultaneous estimation by HPLC from capsule formulation. According to the ICH guidelines recommendations the experimental was validated and USP-30 for parameters such as, system suitability, accuracy, precision, linearity and specificity.

3.6. System suitability: System suitability parameters like resolution, retention time, tailing factor and column theoretical plates was performed by injecting six replicates of standards and two replicates of sample preparation at a 100% level to cross verify the accuracy and precision of the chromatographic system.

3.7.Linearity: The chromatographic method linearity was established by plotting a graph to concentration vs peak area of AML andPEAstandard and determining the correlation coefficients (R2) of the two compounds. For the linearity studies of 2-10 µg/ml of AML, 3-15µg/ml of PEArespectively was injected into the HPLC system. For 60 minutes column was equilibrated with the mobile phase before injection of the solutions.

3.8. Accuracy: The recovery experiments show the accuracy of the method. The recovery was performed by adding AML andPEAworking standards to placebo (excipients mixture) in the range of test concentration (60%, 80% and 100 %) and expressed as percent (%) recovered. Three samples were prepared for each recovery level. The recovery statistical results are within the acceptance range (S.D. < 2.0) value for AML andPEA.

3.9. Precision: In the proposed method **t**he intraday and interday precision was determined by analyzing the sample responses 4 repeats on the same day and 4 different days of a week for 4 different concentrations of standard solutions of AML and PEA. 4-10 µg/mlof AML and6-15µg/ml of PEArespectively and results are represented in terms of % RSD.

3.10. Specificity: The analytical method specificity is to measure the compound accurately in presence of interferences like excipients, degradants and matrix components. TheHPLC of standard mixture and formulation shows specificity of method. TheHPLC method is able to access the analyte in presence of excipients.

3.11. Statistical Parameters: The results of assay obtained are subjected to the following statistical analysis, standard deviation, relative standard deviation, coefficient of variation and standard error.

IV. Results And Discussion

The HPLCchromatogram of AML andPEAare presented in figure 1,2. Wavelength270nm was selected by scanning all standard drugs over a wide range of wavelength 200-400nm. Linearity was evaluated by plotting peak area as a functional of analyte concentration for AML andPEA. The graphical representation was given in figure 3 and4 data is presented in table 1.

The system suitability parameters like resolution, tailing factor, retention time and theoretical plates for the developed RP-HPLC method data are presented in table 2. The limit of detection and limit of quantification for AML and PEA are presented in table 3.

The specific range was determined from linearity studies, for both drugs and found to be 2-10 µg/ml of AML and 3-15 µg/ml of PEA. The data was analyzed by linear regression least square fit method. The slope, intercept, correlation coefficient and regression equation were also determined and the data presented in table 4.

TheAML andPEAchromatographic retention time found to be 4.2 and 7.3 minutes respectively. This is well within the specific limits of 15 minutes. The high – resolution of AML andPEAindicates complete separation of the drugs. The tailingfactor was found to be 0.769 and 0.780 for AML andPEArespectively. The peaks are symmetrical and theoretical plates for AML andPEAwere 7065, and 7952 respectively, which shows the column efficient performance. The quantitative estimation of AML andPEA tabletformulation was carried out by RP-HPLC method using Methanol: Phosphate buffer (27:73 v/v) using C18 column as the stationary phase.Chromatogram ofAML andPEA tablet formulation shown in the figure 5.Quantitative estimation (Assay) data of AML andPEApresented in table 5.Recovery studies of AML andPEA tablet formulation shown in table 6.

The tablet formulation shows percentage purity ranging from 99.16 to 100.18% for AML and99.58 to 100.23% for PEA.The mean percentage purity is 100.01% and 100.08% forAML andPEArespectively. The percentage deviation was found to be -1.0 to +1.0% and -0.6 to +0.7, for AML andPEArespectively. The RSD values are below 2% indicating the method precision and the accuracy of the method shown by the low standard error values. This shows a good index of accuracy and reproducibility of the developed method. All the parameters including flow rate, detection wavelength sensitivity was maintained constant.

V. Figures And Tables

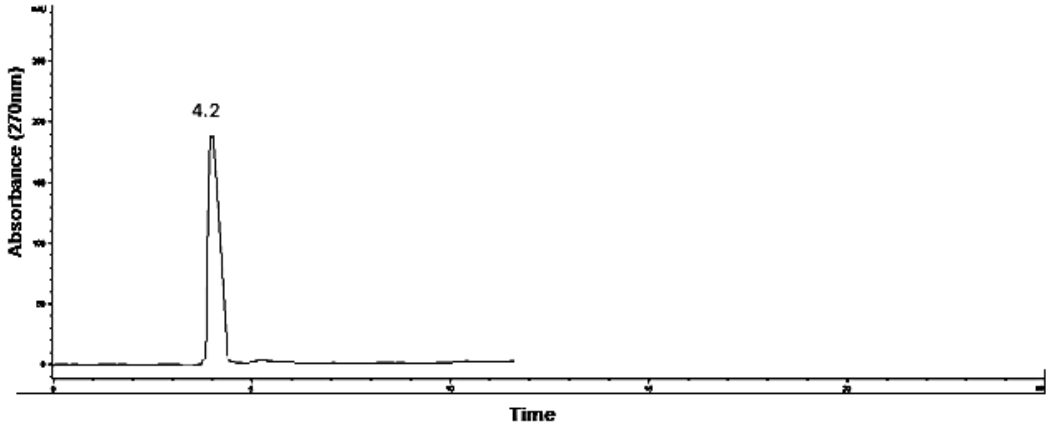

Figure 1:A Typical Chromatogram of Amlodipine Standard

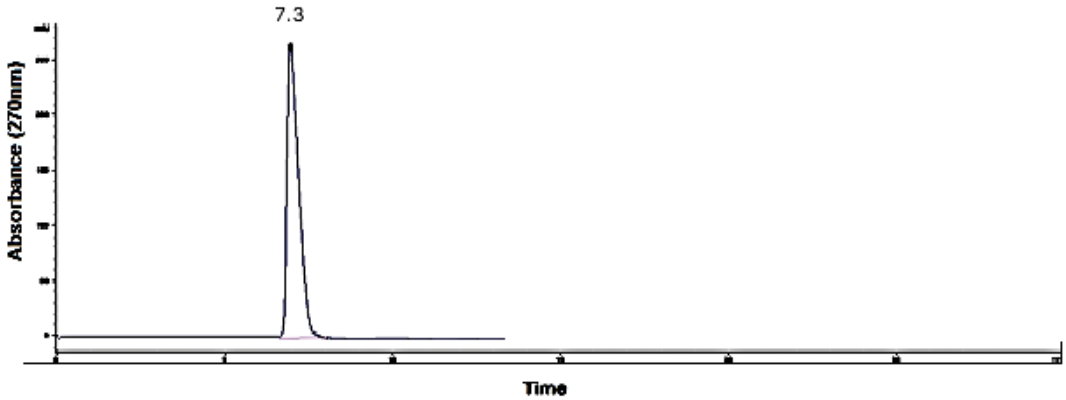

Figure 2: A Typical Chromatogram of Perindopril arginineStandard

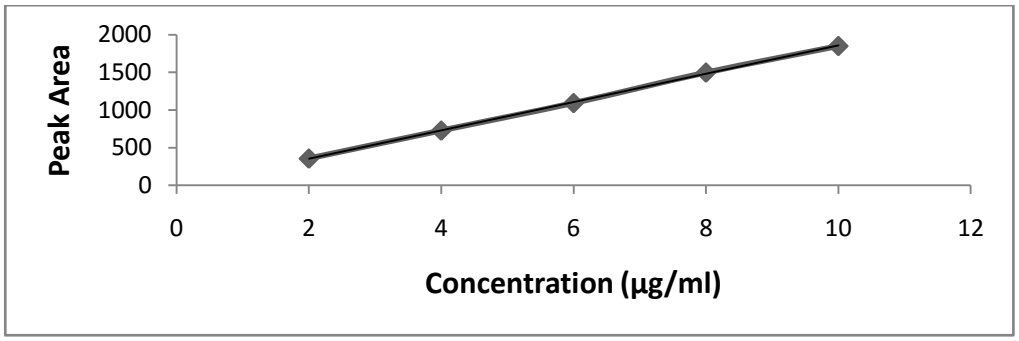

Figure 3: Calibration graph of Amlodipine 2-10µg/ml precision

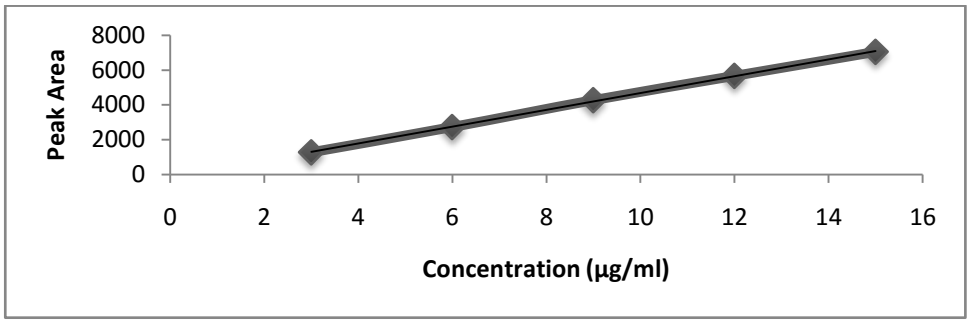

Figure 4: Calibration graph of Perindopril arginine3-15 µg/ml precision

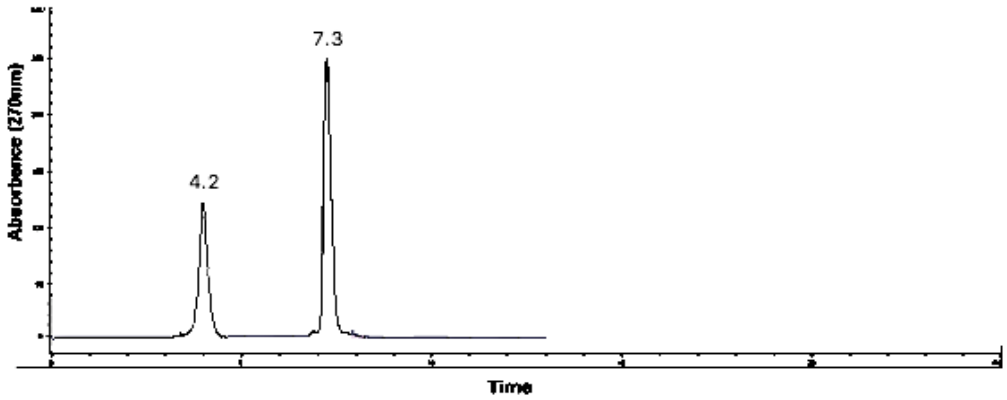

Figure 5: Chromatogram of Amlodipine and Perindoprilintablet formulation

SNo	Concentration (µg/ml) of Amlodipine	Peak area	Concentration (µg/ml) of Perindopril	Peak area
1	2	357.25	3	1274.75
2	4	727.75	6	2728.85
3	6	1093.27	9	4273.37
4	8	1497.45	12	5667.75
5	10	1849.69	15	7049.69

Table 1:HPLC linearity data for Amlodipine and Perindopril

SNo	Parameters	Amlodipine	Perindopril
1.	Theoretical plates	7065	7952
2.	Tailing factor	0.769	0.780
3.	Resolution factor	9	9
4.	Retention time	4.2	7.3
5.	Calibration range or Linear dynamic range	2-10	3-15

Table 2: Results of system suitability parameters

Parameters	Amlodipine	Perindopril
LOD(µg/ml)	0.330	0.500
LOQ(µg/ml)	1.080	1.300

Table 3: Results of Limit of detection (LOD) & limit of quantification LOQ

SNo	Parameters	Amlodipine	Perindopril
1.	Standard deviation (SD)	6.73	4.78
2.	Relative standard deviation (RSD)	0.00616	0.0142
3.	% RSD	0.616	1.421
4.	Standard error (SE)	0.02476	0.01305
5.	Correlation Coefficient (r)	0.9897	0.9794
6.	Slope (a)	43.591	27.323
7.	Intercept (b)	16.106	10.114
8.	Regression equation Y = (a X + b)	Y = 43.591 X + 16.106	Y = 27.323 X +10.114

Table 4: Results of statistical parameters Statistical parameters

S No	Drug	Label claim (mg/Tab)	Amount found (mg/Tab)	Mean amount found (mg/ Tab)	Percentage purity (% w/w)	Mean percentage purity (% w/w)	% Deviation
1.	AML	2.5	2.57 2.68 2.54 2.34 2.52	2.53	100.07 100.18 100.04 99.16 100.02	100.01	+ 0.7 +0.18 +0.4 -0.5 +0.2
2.	PEA	3.5	3.42 3.57 3.73 3.60 3.56	3.57	99.58 100.07 100.23 100.10 100.06	100.08	-0.5 +0.7 +0.2 +0.1 +0.6

Table 5: Quantitative estimation (Assay) data of Amlodipine and Perindopril

S No	Drug	Amount of Drug present in preanalyzed Sample	Amount of Standard drug (RS) added (µg/ml)	Amount of drug recovered (µg/ml)	% Recovery	Mean recovery in Percentage
1.	AML	6	4.00 6.00 8.00	10.33 12.24 14.72	100.13 100.78 99.96	100.22
2.	PEA	12	6.00 12.00 16.00	18.32 24.68 28.74	99.92 100.73 100.77	100.38

Table 6: Recovery studies of Amlodipine andPerindopriltablet formulation

VI. Conclusion

The proposed and developed RP-HPLC method is precise, accurate, and sensitive. The method is rapid, reproducible, and economical and does not have any interference due to the excipients in the pharmaceutical preparations.

Acknowledgement

The authors are thankful and acknowledge Jazan University for required facilities to carry out this research work.

References

[1]. *Amlodipine: MedlinePlus Drug Information"www.nlm.nih.gov*, Retrieved 2015-.
[2]. Cash, Jill C.; Glass, Cheryl Anne, Family Practice Guidelines, Third Edition, 2014.
[3]. JP. Bounhoure, G. Bottineau andP.Lechat, Value of perindopril in the treatment of chronic congestive heart failure: multicentredouble-blind placebo-controlled study,*ClinExpHypertens*, 11(2), 1989, 575-586.
[4]. Indian Pharmacopoeia–2007, Govt. of India Ministry of Health and Family Welfare, The Indian Pharmacopoeia Commission, Ghaziabad, Vol 2 & 3, pp- 745-746, 1676-1677.
[5]. British Pharmacopoeia (BP) 2008 The Stationery Office. London, UK, Vol I, pp -37-138,1690-1692.
[6]. C. Arauz-Pacheco, MA.Parrott and P. Raskin, The treatment of hypertension in adult patients with diabetes, *Diabetes Care, 25*, 2002, 134–147.
[7]. P. Gaurav, P. Sanjay, P. DharmeshandM. Rajendra, RP-HPLC Method for Simultaneous estimation of Amlodipine Besylate and Hydrochlorothiazide in Combined Dosage Forms,*Stamford Journal of Pharmaceutical Sciences*,*3*,2010, 453-462.
[8]. V. Amudhavalli, K. Lakshmi and M. Karthick, Determination of Olmesartan and Hydrochlorthiazide in pharmaceutical formulations by RP-HPLC,*Int J ChemSci, 9*,2011, 470-476.
[9]. GK. Aulakh, RK. Sodhi and M. Singh, "An update on non-peptide angiotensin receptor antagonists and related RAAS modulators", *Life Sci. 81 (8)*, 2007, 615–639.
[10]. G. Saravanan, D. Kommara, I.S. Krishnanjaneyulu, D.Visagaperumal, Stability indicating RP- HPLC method for simultaneous estimation of Amlodipne and Perindopril in bulk and formulation, *J. Chem. Pharma. Res*,6, 2014, 465 – 473.
[11]. P. Patil, H. More,S.Pishwikar, RP-HPLC for simultaneous estimation of Amlodipine besylate and Olmesartanmedoxomil from tablet,*Int J Pharm PharmSci, 3*,2011, 146-149.

[12]. C. Rao, K. Kakumani, V. Maddala, S. Polisetty, M. Gutta and M.Khagga,Development and validation of stability indicating LC method for Olmesartanmedoxomil,*Am J Anal Chem,3,*2012, 153-160.
[13]. PD. Bari, AR. Rote, RP-LC and HPTLC methods for the determination of Olmesartanmedoxomil and Hydrochlorothiazide in combined tablet dosage forms,*Chromatographia 69,*2009, 1469-1472.
[14]. A. Chabukswa, B. Kuchekar, S. Jagdale, D. Mehetre, A. More andP. Lokhade, Development and validation of a RP-HPLC method for simultaneous estimation of Olmesartanmedoxomil and Amlodipinebesylate in tablet dosage form,*Arch ApplSciRes,2,*2010, 307-312.
[15]. P.Gaurav, P. Sanjay,P.Dharmesh,M.Rajendra, RP-HPLC Method for Simultaneous estimation of Amlodipine Besylate and Hydrochlorothiazide in Combined Dosage Forms. *Stamford Journal of Pharmaceutical Sciences,3,*2010, 453-462.
[16]. G Manoharan, M. Bratty. Development and validation of Reversed-phase HPLC method for the simultaneous estimation of amlodipine and nebivololin raw and tablet formulation, *International journal of pharmaceutical research and allied sciences, 5(4),* 2016,51-58.
[17]. PS. Jain, MK, Patel, AP. Gorle,AJ. Chaudhari, SJ. Surana, Stability-Indicating method for simultaneous estimation of Olmesartanmedoxomil, Amlodipine Besylate and Hydrochlorothiazide by RP-HPLC in tablet dosage form,*JChromatogrSci,50,* 2012, 680-687.
[18]. KK. Kakumani,KR.Chimalakonda,G.Madhusudan,M.Khagga, Rapid simultaneous determination of Olmesartan, Amlodipine and Hydrochlorothiazide in combined pharmaceutical dosage form by Stability-Indicating ultra performance liquid chromatography.*Am J Anal Chem.* 2012; 3: 50-58.
[19]. MB. Neela, JD. Snehal, NM. HarinathandBC. Prafulla, Simultaneous Spectrophotometric estimation of the Amlodipine Besylate and Hydrochlorothiazide.*Asian J Res Chem, 2,*2009, 393-397.
[20]. KR.Patil, VP. Rane, JN. Sangshetti, RD. Yeole, DB.Shinde, Stability indicating LC method for the simultaneous determination of Amlodipine and Olmesartan in dosage form,*JChromatogrSci,48,* 2010, 601-606.
[21]. D.S. Jain, G. Subbaiah, M. Sanyal, U.C. Pande and P. Shrivastav, First LC–MS/MS electrospray ionization validated method for the quantification of perindopril and its metabolite perindoprilat in human plasma and its application to bioequivalence study, J. *ChromatogrB,837,* 2006, 92 – 100.