Available Online at http://www.journalajst.com

ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN: 0976-3376

Asian Journal of Science and Technology
Vol. 14, Issue, 01, pp.12386-12390, January, 2023

RESEARCH ARTICLE

METHOD DEVELOPMENT AND VALIDATION FOR ANALYSIS OF RESIDUAL SOLVENTS IN BUDESONIDE BY HS-GC

Noel Mankoo[1], Navjit Kaur[2], Lakhwinder Singh[3], Monika Gupta[4], Tejinder Kaur[5] and Divya Dhawal Bhandari[6]*

[1,2,3,4] ASBASJSM College of Pharmacy, (An Autonomous College), Bela(Ropar)-140111, Punjab, India
[5]University Institute of Pharma Sciences, Chandigarh University, Gharuan (Mohali)-140103, Punjab, India
[6]University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh-160014, India

ARTICLE INFO

Article History:
Received 19th October, 2022
Received in revised form
20th November, 2022
Accepted 19th December, 2022
Published online 30th January, 2023

Keywords:

Robustness, Chromatogram, Specificity, Precision, Linearity and Range.

ABSTRACT

Residual solvents are organic volatile impurities which are harmful to environment as well as human health. The analysis of such solvents is an important part in maintaining the quality of drug. A number of analytical methods are available for such analysis but most of them are restricted to low detection limits or lack of accuracy. Gas chromatography is one of the widely used method for such analysis due to more accuracy and high detection limits. The present study is focused on the analysis of residual solvents in Budesonide drug by HS-GS Chromatography by development and validation of a suitable method. The residual solvents methanol, acetone, methylene chloride, di-isopropyl ether and butraldehyde were determined. Based upon analytical data and result of each study it is concluded that the method is specific, precise, accurate, linear, robust and rugged with established limit of detection and limit of quantitation. The % RSD of 50 % level of all above solvents are 93.57, 91.07, 91.64, 102.11 and 93.07. The percentage recovery for all above solvents are between 80% and 120%, which is well within the acceptance criteria. Hence, accuracy by recovery for the solvents was established. The relative standard deviation between results of sample obtained with changed condition and that under normal experimental condition is within the acceptance criteria.

Citation: ***Noel Mankoo, Navjit Kaur, Lakhwinder Singh, Monika Gupta, Tejinder Kaur and Divya Dhawal Bhandari.*** **2023.** "Method development and validation for analysis of residual solvents in budesonide by hs-gc", *Asian Journal of Science and Technology*, 14, (01), 12386-12390.

INTRODUCTION

The organic volatile impurities being produced during manufacturing of any AIP or excipient or to prepare medicinal products are considered as residual solvents in drugs. These residual solvents acting as chemical residues may be harmful to environment as well as to human health also so the manufacturers of drugs always ensure that such residues should not be present in the product or if present, they must be below acceptable level (1,2,3). The analysis of such residual solvents is important as a part of quality control of drugs being used for clinical studies, pre-clinical studies and commercial drugs. Analytical chemistry is one type of science based on the measurements of chemical compounds or composition of various natural as well as artificial materials with the help of some improved methods(4, 5). Analytical methods are often classification as being either classical or instrumental (5, 6).

Corresponding author:** ***Divya Dhawal Bhandari,
University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh-160014, India.

The older method for such residual solvent analysis included weight loss on heating but that require several grams of product to get detection limit of about 0.1%. Infrared spectroscopy (IR) and Fourier Transform Infrared Spectrometry (FTIR) were also used in determining the residual solvents by the measurement of specific solvent bands in the spectra. The limiting factors in Infrared spectroscopy (IR) and Fourier Transform Infrared Spectrometry (FTIR) methods are possible interferences of solvent and matrix peaks, the high detection limit (above 100 *ppm*) and a lack of accuracy at low concentrations (1, 2). The above mentioned methods can be easily replaced by Chromatography. Chromatography is a technique being used for the separation of various compounds from a mixture. The mixture is solubilized in mobile phase and allowed to pass over a stationary phase and the components of mixture travel at different speed in the mobile phase and thus got separated. Such separation is entirely based on differential partitioning of components between mobile and stationary phase (7, 8,9). Among various chromatographic techniques, GC is the best adopted method for residual solvent analysis. Gas chromatography (GC), is used in analytical chemistry to

separate and analyse compounds that can be vaporized easily without undergoing decomposition. The advantages of GC for residual solvent analysis for other chromatographic methods are:

- Excellent separation ability
- Varying chromatographic conditions
- Differential column
- Low detection limits
- Possible to analyse liquid or solid samples of complex nature.

The mobile phase in GC is a carrier gas (an inert gas such as helium or an unreactive gas such as nitrogen). The stationary phase is a microscopic layer of liquid or polymer on an inert Solid support, inside a piece of glass or metal tubing called a column (a homage to the fractionating column used in distillation). The instrument used to perform gas chromatography is called a gas chromatograph (10-12). The most commonly used detectors for GC are FID and ECD. FID or the flame ionization detector is the one universal detector for organic volatile compounds while ECD or the electron capture detector (ECD) is especially designed for the detection of halogenated compounds. FID is the most preferred one due to its low detection limits, wide linear dynamic range, robustness, ease of operation, general reliability and utility for trace organic compounds (1, 3).

MATERIALS AND METHODS

Instrument and Chromatographic conditions: The type of instrument used and the conditions under which chromatogram is obtained are described in Table 1.

Table 1. Instrument and chromatographic conditions

Instrument detector	Gas chromatograph is equipped with FID
Column	DB-624, 30M x 0.53mm x 3µm
Column temperature Programmed	40°C for 5 minutes, then raised at a rate of 30°C per minute to 220°C,and maintained at 200°C for 10 minute.
Injector temperature	220°C
Detector temperature	250°C
Carrier gas	Nitrogen
Linearity velocity	About 40 cm per second
Split ratio	10
Attenuation	-6
Injection volume	1min
Column flow	2.0 ml/min
GC cycle time	18 min
Incubation temperature	100°C
Incubation Time	15 minutes
Column pressure	20 psi
Withdrawal time	0.2 minutes
Transfer line	110°C
Pressurize Time	1.0 minute.
Needle Temperature	110°C
Injection Volume	0.08 min
GC cycle time	36.7 min

Solvent Details: All the solvents used were of GC grade. Dimethylsulphoxide, Methylene chloride, Di-isopropyl ether and Butraldehyde were procured Mercks Chemical, Acetonitrile from Sigma Aldrich and Methanol from Spectrochem.

Solution preparation

Solution A: Weighed accurately 0.400 g of methanol, 0.164 g of acetonitrile, 0.250 g of methylene chloride, 0.200 g of di-isopropyl ether and 0.200 g of butraldehyde in 50 ml of volumetric flask containing about 20 ml of diluent, diluted up to the mark with same and mix well.

Standard stock solution: Taken 10 ml of solution A in 200 ml volumetric flask. Diluted up to the volume with diluents.

Standard solution: Pipetted out 10 ml of standard stock solution into 100 ml volumetric flask diluted up to the mark with diluents and mix well. Pipetted out 5 ml of standard solution into six vials separately and seal the vials with aluminium seal followed by septa.

Test solution: Weighed accurately 0.200 g of test sample and transfer in 20 ml HSS vial, add 5 ml of diluents seal the vials with septum. Prepared sample solution in duplicate.

Procedure: Before starting the analysis conditioning of the GC column at 200° C for 30 minutes. Inject the sample as per following sequence.

- Inject diluents as blank in singlet.
- Inject standard solution in six replicate.
- Inject test solution in duplicate.

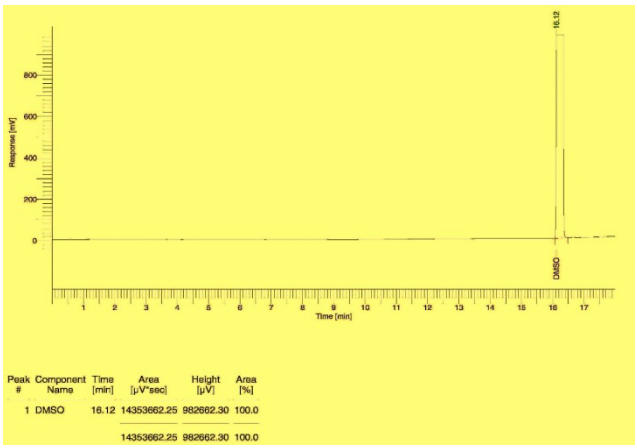

Fig. 1. Chromatogram for Blank

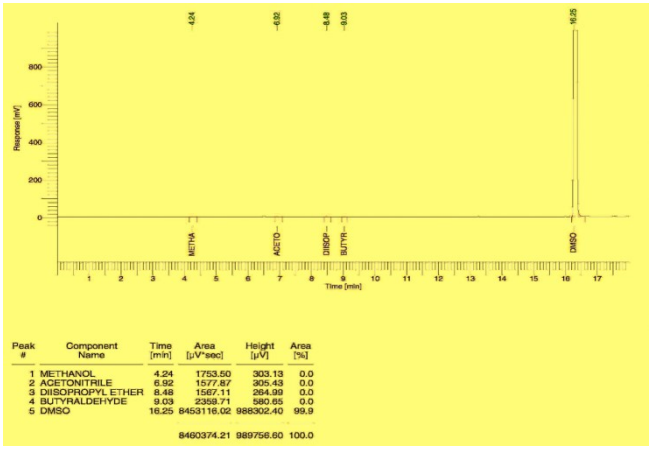

Fig. 2. Chromatogram for Sample

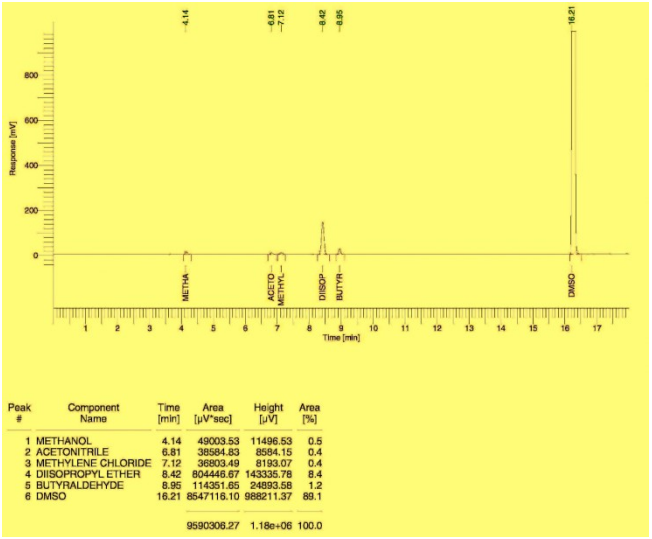

Fig. 3 Chromatogram for Standard

RESULT AND DISCUSSION

In this study, the method was developed after various trails and further validated in terms of specificity, linearity, precision, accuracy and robustness. The validation was carried out in compliance with the ICH-Guidelines Q2 (R1) and 12/QC1034. The objective of this validation was to demonstrate that the test method used is suitable for its intended purpose. The percentage of residual solvent was calculated in *ppm* by using the mean area of six replicate injection of standard solution.

The retention time of solvents was as follows. Methanol is about 4.30, Acetonitrile is about 7.00, Methylene Chloride is about 7.30, Di-isopropyl ether is about 8.60 and Butraldehyde is about 9.10

Calculation

$$= \frac{AT}{AS} \times \frac{WS}{WT} \times D \times P \times 1000000$$

Where,

AT = Area of solvent in test solution.
AS = Average area of solvent in standard solution.
WS = Weight of standard solvent in g.
WT = Weight of test sample in g.
D = Dilution factor 0.0005.
P = Purity of respective standard solvent.

The RT of allsolvents are about 4.15 min, 6.83 min, 7.13, 8.43, 8.76 and RRT are about 1.00, 1.65, 1.72, 2.03 and2.16. There are no interference peaks from blank at the retention time of standard peaks and observation is no interference observed at the RT of all solvents.
Hence the method is specific. Correlation coefficient for each solvent should be NLT 0.99. The % recovery of solvents should be between 80% to 120%. The overall mean of all solvents are about 95.99%, 92.45%, 92.83%, 102.83%, 93.46%. Hence the accuracy by recovery for the solvents was established.Validation results show satisfactory precision, specificity, accuracy, linearity, robustness and stability of solutions which were found to be passing all the acceptance criteria. (13-18). Samples were separated by DB-624, 0.53 mm x 30 m x 3µ capillary column with carrier gas nitrogen and split ratio is 10. Statistical data of the method is summarized below.

Specificity: It is the ability to assess unequivocally the analyte in the presence of components that may be expected to present.

Table 2. Summary of validation parameters for the proposed method

Specificity	No interference peaks from blank at the Retention time of standard peaks.
Robustness	% RSD between results of sample obtained with changed condition and that under normal experimental condition is within the acceptance criteria (15%).
Linearity (R)	0.99
Accuracy (% recovery)	1.23-2.66
Ruggedness	% RSD for each solvents peak area are within the limits (12.10% - 13.16%)
LOD	% RSD for each solvent found to be within limits.(5.61-15.75, <33%)
LOQ	% RSD for each solvent found to be within limits.(3.02-7.41, <15%)
Range Precision (RSD) System Precision Method precision Intermediate Precision	 2.19-3.33 7.67-8.52 8.16-10.89

Linearity and range: Linearity can be defined as the response of an analytical method conducted as a function of the analyte concentration. In other words, it is the response of an analytical method in proportion to the concentration of analyte in samples.

Precision: The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.The % RSD for standard solution should not be more than 15 %. (Table 3)

Table 3. Percentage relative standard deviation of precision

Me (% RSD)	Ace (% RSD)	Mec (% RSD)	Dip (% RSD)	But (% RSD)
3.29	3.33	2.33	2.19	2.39

Table 4. % relative standard deviation of intermediate precision

Me (% RSD)	Ace (% RSD)	Mec (%RSD)	Dip (% RSD)	But (% RSD)
10.89	8.16	0.00	0.00	0.00

Limit of Detection: The % RSD for each solvent is not more than 33% for LOD (Table 5).

Table 5. Percentage relative standard deviation of limit of detection

Me (% RSD)	Ace (% RSD)	Mec (% RSD)	Dip (% RSD)	But (% RSD)
15.75	15.29	5.27	3.08	5.61

Limit of Quantitation: The % RSD for each solvent is not more than 10% for LOQ. (Table 6).

Linearity and Range: Correlation Coefficient for each solvent should be NLT 0.99.

Table 6. Percentage relative standard deviation of limit of quantitation

Me (% RSD)	Ace (% RSD)	Mec (% RSD)	Dip (% RSD)	But (% RSD)
7.41	6.49	2.95	4.79	3.02

Table 7 Percentage relative standard deviation of accuracy by recovery

Me (% RSD)	Ace (% RSD)	Mec (% RSD)	Dip (% RSD)	But (% RSD)
2.15	1.47	1.23	2.66	1.59

Table 8. System Suitability Parameters

Robustness	Me (% RSD)	Ace (% RSD)	Mec (% RSD)	Dip (% RSD)	But (% RSD)
Normal condition (40°C)	5.08	4.20	4.69	8.49	3.99
Temperature minus (36°C)	2.45	3.04	3.04	5.09	3.17

Correlation Coefficient (R^2) for methanol, acetonitrile, methylene chloride, diisopropylether and butraldehyde is 0.999, 0.991, 0.999, 0.997 and 0.996 respectively.

Accuracy (By Recovery): The % RSD of replicate injection from standard solution should not more than 15% for area. (Table 7)

Robustness comparative data: System suitability parameters achieved in normal condition and robust condition (Temperature minus). From the results obtained it is clear that the method validation data meets the acceptance criteria of USP and ICH Q2 R1 guidelines.

Linearity and range: Linearity can be defined as the response of an analytical method conducted as a function of the analyte concentration. In other words, it is the response of an analytical method in proportion to the concentration of analyte in samples.

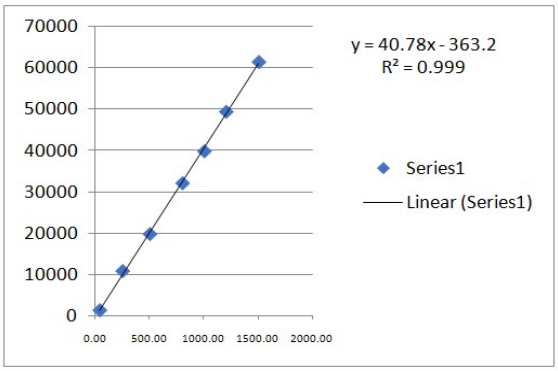

Fig.4 Linearity of Methanol

Table 9 Linearity of Methanol

Level	Conc.	Mean Area
LOQ	40.03	1210
25%	251	10717
50%	502	19653
80%	803	31992
100%	1004	39718
120%	1204	49298
150%	1505	61364
Slope	40.7836	
Intercept	-363.2501	
r^2	0.9996	

Calculation

$$PPM = \frac{\text{Wt. of std (mg)}}{50} \times \frac{10}{200} \times \frac{\text{Volume of stock solution}}{50} \times 1000000$$

Table 10. Linearity of Acetonitrile

Level	Conc.	Mean Area
LOQ	9.96	3436
25%	104	13301
50%	207	16295
80%	332	25556
100%	415	31016
120%	498	37972
150%	622	47252
Slope	68.933	
Intercept	3430.5133	
r^2	0.9955	

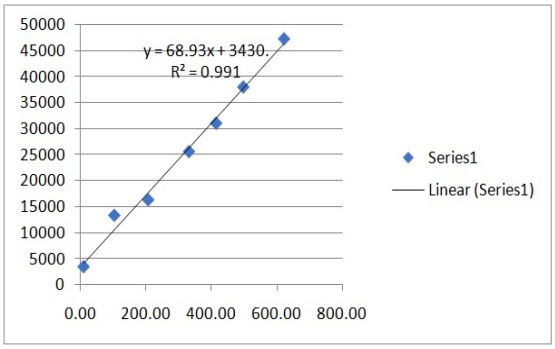

Fig. 5 Linearity curve for acetonitrile

Table 11. Linearity of Methylene Chloride

Level	Conc.	Mean Area
LOQ	40.17	956
25%	151	7425
50%	301	14263
80%	482	22998
100%	603	29097
120%	723	35819
150%	904	44855
Slope	50.2918	
Intercept	-823.3684	
r^2	0.9997	

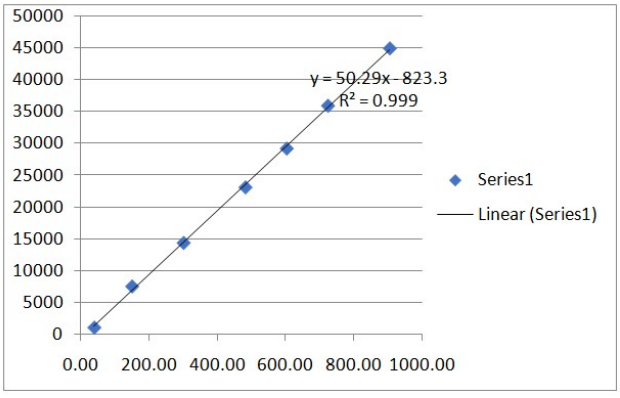

Fig. 6. Linearity curve for Methylene chloride

Table 12. Linearity of Di-isopropyl ether

Level	Conc.	Mean Area
LOQ	10.16	12743
25%	148	207348
50%	296	417135
80%	474	619203
100%	592	856202
120%	710	1018528
150%	888	1298573
Slope	**1457.4294**	
Intercept	**-16418.0454**	
r^2	**0.9985**	

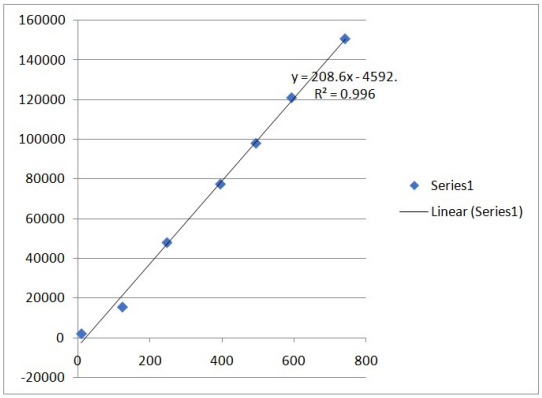

Fig. 8. Linearity curve for Butraldehyde

Table 14. Linearity and accuracy of residual solvents

	Linearity			Accuracy			
Solvents	Range (%)	R^2	Slope	Recovery (%)	Average value (ppm)	Average	RSD (%)
Methanol	0.04-8.0	0.9985	32.02	97.66-101.34	3028.35	99.54	5.26
Ethanol	0.02-8.0	0.9988	46.25	98.60-100.76	5093.04	99.01	4.72
Acetone	0.05-8.0	0.999	178.79	93.09-96.65	4770.95	94.65	5.63
Acetonitrile	0.01-0.8	0.999	186.43	98.72-101.55	420.95	99.21	5.47
Toluene	0.04-8.0	0.991	492.56	92.86-95.80	846.54	93.89	4.81

Conclusion

Residual solvents are determined in pharmaceutical products in a single run using static headspace GC. All the residual solvents used in this method validation are class 2 and class 3 solvents which can cause toxicity to the body. Solvents including water are used in almost every step of the elaboration of a drug product. Their residues could be detrimental for the processibility and stability of the pharmaceutical products and the safety of the patients. The testing and control of residual solvents has thoroughly assessed and is based on robust and sensitive techniques. There are limitations known for other techniques of residual solvents determination such as many drugs react with Karl Fischer reagents in Karl Fischer titration. The result obtained with loss on drying technique differs widely from accepted results. Thermo gravimetric has low sensitivity and in infrared spectroscopy, the water bands for low water content solutions are not easily observed. The techniques mentioned above are now a days replaced by GC. A single, rapid and highly selective GC method was developed and validated for the quantification of residual solvents present in Budesonide through an understanding of the synthetic process, nature of solvents and nature of stationary phases of columns. A number of trials were taken for selection of diluent, column, temperature, split ratio and flow rate.

The specificity obtained indicates non-interference from the solvents present in Budesonide. The residual solvents methanol, acetone, methylene chloride, di-isopropyl ether and butraldehyde were determined. Then, based upon analytical data and result of each study it is concluded that the method is specific, precise, accurate, linear, robust and rugged with established limit of detection and limit of quantitation. The % RSD of 50 % level of all above solvents are 93.57, 91.07, 91.64, 102.11 and 93.07. The percentage recovery for all above solvents are between 80% and 120%, which is well within the acceptance criteria. Hence, accuracy by recovery for the solvents was established. The relative standard deviation between results of sample obtained with changed condition and that under normal experimental condition is within the acceptance criteria. Hence it is concluded that method is robust. The amount of all organic volatile impurities present in Budesonide was found to be within the ICH limits. Hence the proposed GC method is simply recommended for the quality control of raw material.

Conflict of interest: The authors declare no conflict of interest.

Acknowledgement: Authors would like to thank ASBASJSMCOP, Bela for providing necessary facilities.

REFERENCES

Rele, R. V. R.N. Mali. Am. J. Pharm. Tech. 2 (2012) 4.
Puranik, S.B. R.V. Pawar, N. Lalitha, G.K. Rao. Pak. J. Pharm. Science. 22 (2012) 410.
Jahnavi, N. V.S. Saravanan. Int. J. Res Pharm. Chem. 2 (2012) 2231.
Kumar, V.A. G. Aravind, I. Srikanth. Der. Pharma. Chem. 4 (2012) 2228.
Lokhande, R.S. P.U. Singare, P.V. Yadav. Am. J. Chem. 2 (2012) 1.
Kashikar. V.S. Int. J. Chem. Res. 2 (2011) 32-34.
Kallemullah, T. M. Ahmed, H.K. Sharma. Der. Pharm. Chem. 3 (2011) 390.
Jat, R.K. A. Shukla, P. Sharma, Y. Patel. Int. J, Pharm. Sci. Res. 2 (2011) 1270.
Pandey, S. P. Pandey, R. Kumar, N.P. Singh. Braz. J. Pharm. Sci. 47 (2011) 380.
Raj, V.V. G. Pramod, N.N. babu. Int. J. Res. Pharm. Biomed. Sci. 1 (2010) 2.
Narkhede, D.B. P.D. Narkhede. Int. J. Pharm. Biomed. Sci. 1 (2010) 2.
Puranik, S.B. R.V. Pawar, N. Lalitha, PN Sanjay Paiand GK Rao. Pak. J. Pharm. Sci. 22 (2009) 410
Reddy, P.B. M.S. Reddy. Int. J. Pharm. Res. 1 (2009) 230.
Groman, A. E.U. Stolarczyk, W. Maruszak. ActaPolinaePharmaceutica. 65 (2008) 783.
Windhorst, A.D. R.P. Klok. J. Pharm. Sci.1 (2006) 344.
C.B. Hymer. J. Chromatograph. Sci. 46 (2008) 369.
Hoonjoo Kim, Dongmin Kim, Jeong Soo Yang. Bull. Korean. Chem. 27 (2006) 302.
Coran, S. A. S. Furlanetto. Int. J. Pharm. Bio Sci. 32 (2005) 30.

****