International Journal of Pharmacy and Industrial Research (IJPIR)

ISSN:2231-6567

IJPIR |Volume 13 | Issue 3 | July - Sept - 2023
Available online at: www.ijpir.com

Research article ***Sanitation***

A validated rp-hplc method for simultaneous estimation of aspirin and prasugrel in bulk and its pharmaceutical dosage form

Sadanala Pavani Satya*[1], Dr. T.K.V. Kesav Rao[1], Mr. A. Venkateswara Rao[1]

[1]Department of Pharmaceutical Analysis, Pydah College of Pharmacy Patavala, Andhra University, Kakinada, Andhra Pradesh,

***Corresponding Author: Sadanala Pavani Satya**

Published on: September 11, 2023

ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Glipizide and Metformin, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Zorbax C18 (4.6 x 150mm, 5µm) column using a mixture of Methanol: Phosphate Buffer pH 3.9 (55:45v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 255nm. The retention time of the Glipizide and Metformin was 2.061, 2.462 ±0.02min respectively. The method produce linear responses in the concentration range of 1-5µg/ml of Glipizide and 100-500µg/ml of Metformin. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Glipizide, Metformin, RP-HPLC, validation.

INTRODUCTION

Analytical chemistry

Analytical chemistry is a scientific discipline used to study the chemical composition, structure and behaviour of matter. The purposes of chemical analysis are together and interpret chemical information that will be of value to society in a wide range of contexts. Quality control in manufacturing industries, the monitoring of clinical and environmental samples, the assaying of geological specimens, and the support of fundamental and applied research are the principal applications. Analytical chemistry involves the application of a range of techniques and methodologies to obtain and assess qualitative, quantitative and structural information on the nature of matter.

- **Qualitative analysis** is the identification of elements, species and/or compounds present in sample.
- **Quantitative analysis** is the determination of the absolute or relative amounts of elements, species or compounds present in sample.

Structural analysis is the determination of the spatial arrangement of atoms in an element or molecule or the identification of characteristic groups of atoms (functional groups). An element, species or compound that is the subject of analysis is known as analyte. The remainder of the material or sample of which the analyte(s) form(s) a part is known as the matrix.

The gathering and interpretation of qualitative, quantitative and structural information is essential to many aspects of human endeavour, both terrestrial and extra-terrestrials. The maintenance of an improvement in the quality of life throughout the world and the management of resources heavily on the information provided by chemical analysis. Manufacturing industries use analytical data to monitor the quality of raw materials, intermediates and finished products. Progress and research in many areas is dependent on establishing the chemical composition of man-made or natural materials, and the monitoring of toxic substances in the environment is of ever increasing importance. Studies of biological and other complex systems are supported by the collection of large amounts of analytical data. Analytical data are required in a wide range of disciplines and situations that include not just chemistry and most other sciences, from biology to zoology, butte arts, such as painting and sculpture, and archaeology. Space exploration and clinical diagnosis are two quite desperate areas in which analytical data is vital. Important areas of application include the following.

Quality control

(QC) in many manufacturing industries, the chemical composition of raw materials, intermediates and finished products needs to be monitored to ensure satisfactory quality and consistency. Virtually all consumer products from automobiles to clothing, pharmaceuticals and foodstuffs, electrical goods, sports equipment and horticultural products rely, in part, on chemical analysis. The food, pharmaceutical and water industries in particular have stringent requirements backed by legislation for major components and permitted levels of impurities or contaminants. The electronic industry needs analyses at ultra-trace levels (parts per billion) in relation to the manufacture of semi-conductor materials. Automated, computer-controlled procedures for process-stream analysis are employed in some industries.

Monitoring and control of pollutants

The presence of toxic heavy metals (e.g., lead, cadmium and mercury), organic chemicals (e.g., polychlorinated biphenyls and detergents) and vehicle exhaust gases (oxides of carbon, nitrogen and sulphur, and hydrocarbons) in the environment are health hazards that need to be monitored by sensitive and accurate methods of analysis, and remedial action taken. Major sources of pollution are gaseous, solid and liquid wastes that are discharged or dumped from industrial sites, and vehicle exhaust gases.

Clinical and biological studies

The levels of important nutrients, including trace metals (e.g., sodium, potassium, calcium and zinc), naturally produced chemicals, such as cholesterol, sugars and urea, and administered drugs in the body fluids of patients undergoing hospital treatment require monitoring. Speed of analysis is often a crucial factor and automated procedures have been designed for such analyses.

Geological assays[15]

The commercial value of ores and minerals are determined by the levels of particular metals, which must be accurately established. Highly accurate and reliable analytical procedures must be used for this purpose, and referee laboratories are sometimes employed where disputes arise.

MATERIALS AND METHODS

Aspirin from Sura labs, Prasugrel from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK). Acetonitrile for HPLC from Merck,

HPLC METHOD DEVELOPMENT TRAILS

Preparation of standard solution

Accurately weigh and transfer 10 mg of Aspirin and Prasugrel working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 1.5ml of Aspirin and 0.15ml of Prasugrel from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization

Initially the mobile phase tried was water, Methanol: Water with varying proportions. Finally, the mobile phase was optimized to Acetonitrile and water in proportion 35:65 v/v respectively.

Optimization of Column

The method was performed with various columns like C18 column, Symmetry and X-Bridge. Symmetry C18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Instrument used	:	Waters HPLC with auto sampler and PDA, Detector 996 model.
Temperature	:	40ºC
Column	:	Symmetry C18 (4.6×150mm, 5µ)
Mobile phase	:	Acetonitrile: water (35:65% v/v)
Flow rate	:	1ml/min
Wavelength	:	238nm
Injection volume	:	10 µl
Run time	:	7 min

VALIDATION

PREPARATION OF BUFFER AND MOBILE PHASE

Preparation of mobile phase

Accurately measured 350 ml (35%) of Acetonitrile and 650 ml of Water (65%) a were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase : Water: Acetonitrile (65:35)
Column : Symmetry C18 (4.6×150mm, 5.0 µm)
Flow rate : 1 ml/min
Wavelength : 238 nm
Column temp : 40ºC
Injection Volume : 10 µl
Run time : 7 minutes

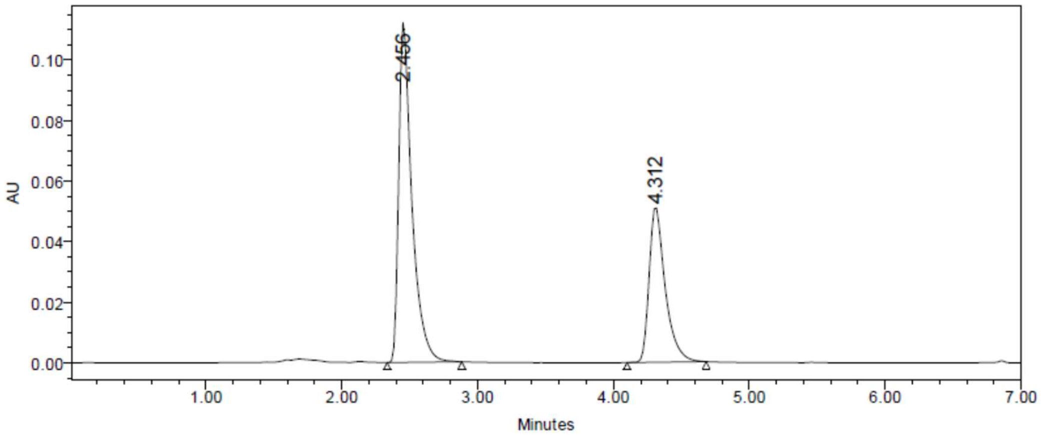

Fig 1: Optimized Chromatogram

Table 1: peak results for optimized

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Aspirin	2.456	600122	112157		1.6	5215
2	Prasugrel	4.312	422042	51068	3.2	1.5	5648

From the above chromatogram it was observed that the Aspirin and Prasugrel peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

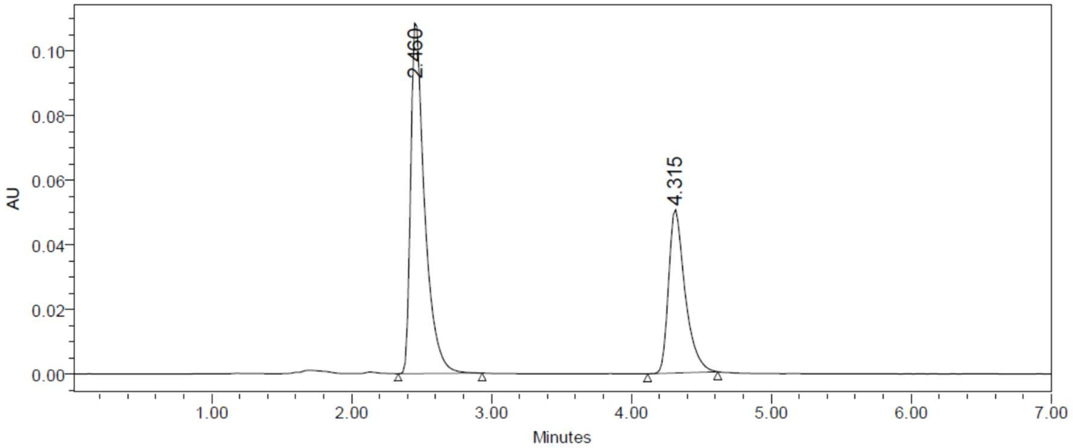

Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Aspirin	2.460	600123	112157		1.6	5011
2	Prasugrel	4.315	422041	51068	3.3	1.5	5947

- *Resolution between two drugs must be not less than 2*
- *Theoretical plates must be not less than 2000*
- *Tailing factor must be not less than 0.9 and not more than 2.*

Assay (Standard)

Table 3: Results of system suitability for Aspirin

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Aspirin	2.459	602561	111160	5123	1.4
2	Aspirin	2.466	600543	53992	5023.2	1.4
3	Aspirin	2.472	601288	55420	5061.3	1.3
4	Aspirin	2.452	600776	112478	5147.3	1.6
5	Aspirin	2.450	600758	111779	5101.8	1.7
Mean			601185.2			
Std. Dev			816.3576			
% RSD			0.13			

- *%RSD of five different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is suitable.*

Table 4: Results of system suitability for Prasugrel

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Prasugrel	4.322	422674	50988	5949	1.5	3.2
2	Prasugrel	4.323	424692	49813	5890.0	1.5	3.3
3	Prasugrel	4.342	421255	49826	5952.5	1.4	3.2
4	Prasugrel	4.300	415235	51804	5926.4	1.50	3.2
5	Prasugrel	4.295	416260	51274	5898.5	1.49	3.2
Mean			420023.2				
Std. Dev			724.7845				
% RSD			0.17				

- *%RSD for sample should be NMT 2*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Assay (Sample)

Table 5: Peak results for Assay sample

S.no	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Aspirin	2.465	601812	110102		1.6	5028	1
2	Prasugrel	4.337	414764	49842	3.2	1.5	5949	1
3	Aspirin	2.474	600435	108333		1.6	5189	2
4	Prasugrel	4.356	418130	48360	3.3	1.5	5818	2
5	Aspirin	2.465	600212	112453		1.6	5061	3
6	Prasugrel	4.337	413645	48641	3.2	1.5	5812	3

$$\%\text{ASSAY} = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

= 600819.7/600180×10/150×150/0.0265×99.7/100×0.2655/100×100
= 99.7%
The % purity of Aspirin and Prasugrel in pharmaceutical dosage form was found to be 99.7 %.

Linearity
Chromatographic data for linearity study
Aspirin

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33.3	50	215760
66.6	100	417001
100	150	600435
133.3	200	791969
166.6	250	974736

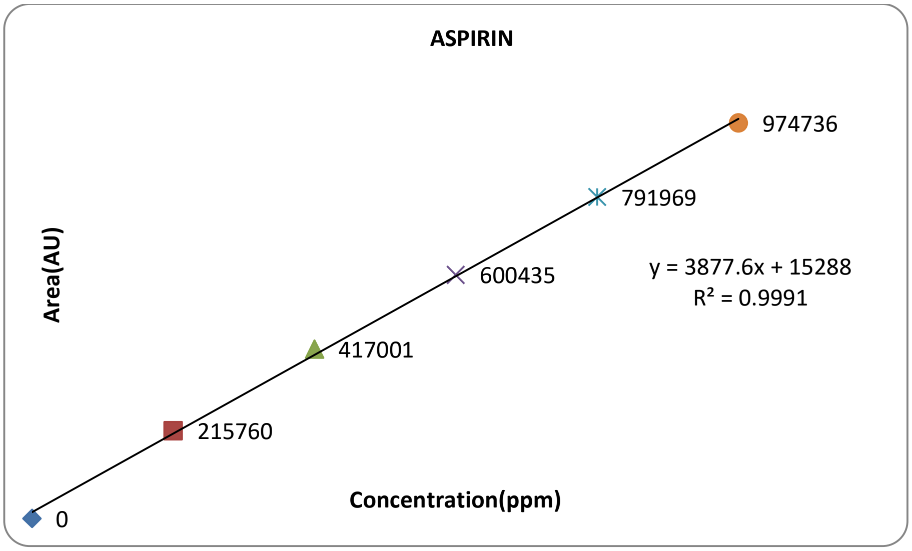

Fig 3: Calibration graph for Aspirin

Prasugrel

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	5	145474
66	10	279372
100	15	421045
133	20	562151
166	25	721671

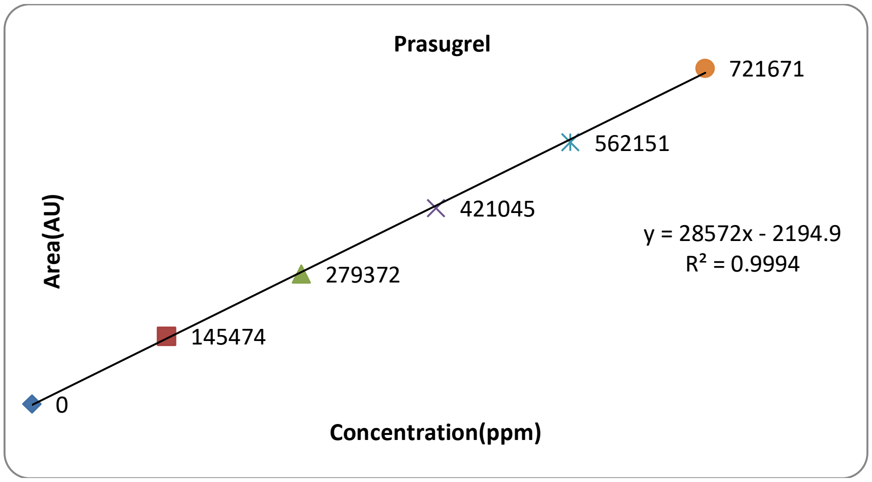

Fig 4: Calibration graph for Prasugrel

Repeatability

Table 6: Results of repeatability for Aspirin

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Aspirin	2.453	603403	112688	5881.3	1.4
2	Aspirin	2.455	608107	113637	5844.1	1.3
3	Aspirin	2.453	607266	112849	5918.1	1.3
4	Aspirin	2.452	608776	112478	5847.3	1.4
5	Aspirin	2.450	609758	111779	5801.8	1.5
Mean			607462			
Std. Dev			2445.82			
% RSD			0.40			

- *%RSD for sample should be NMT 2*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Table 7: Results of method precession for Prasugrel

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Prasugrel	4.289	429183	52411	5050.9	1.49	3.2
2	Prasugrel	4.309	416643	52475	5084.8	1.5	3.2
3	Prasugrel	4.306	424052	51841	5000.1	1.4	3.2
4	Prasugrel	4.300	425235	51804	5026.4	1.51	3.2
5	Prasugrel	4.295	416260	51274	5098.5	1.51	3.2
Mean			422274.6				
Std. Dev			5646.668				
% RSD			1.3				

- *%RSD for sample should be NMT 2*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Table 8: Results of Intermediate precision Day 2 for Aspirin

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Aspirin	2.456	602581	112175	5013	1.7
2	Aspirin	2.457	600985	112422	5007	1.7
3	Aspirin	2.456	600145	114513	5198	1.8
4	Aspirin	2.459	600332	111580	5246	1.7
5	Aspirin	2.467	600566	110347	5096	1.8
6	Aspirin	2.459	600332	111580	5178	1.8
Mean			600823.5			
Std. Dev			908.2622			
% RSD			0.15			

- *%RSD of five different sample solutions should not more than 2*

Table 9: Results of Intermediate precision for Prasugrel

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Prasugrel	4.312	425263	50936	5981	1.5	3.2
2	Prasugrel	4.308	427069	51400	5887	1.49	3.2
3	Prasugrel	4.312	424231	51236	5928	1.5	3.2
4	Prasugrel	4.322	423569	51084	5898	1.50	3.2
5	Prasugrel	4.324	414361	50295	5887	1.5	3.2
6	Prasugrel	4.322	413569	51084	5940	1.5	3.2
Mean			421343.7				
Std. Dev			5841.789				
% RSD			1.38				

- *%RSD of five different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is rugged.*

Accuracy

Table 10: The accuracy results for Aspirin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	308408	75	75.5	100.6	100.3%
100%	600619	150	150	100	
150%	894293	225	226	100.4	

Table 11: The accuracy results for Prasugrel

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	216092	7.5	7.55	100	99.7%
100%	423626	15	14.95	99.6	
150%	634469.7	22.5	22.4	99.5	

- *The percentage recovery was found to be within the limit (98-102%).*

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Aspirin

Table 12: Results for Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	600122	2.456	5215	1.8
Less Flow rate of 0.9 mL/min	651206	2.741	5199	1.79
More Flow rate of 1.1 mL/min	546820	2.270	5234	1.8
Less organic phase	586420	3.266	5298	1.8
More organic phase	542813	2.147	5287	1.76

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Prasugrel

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	422042	4.312	5648	1.5
Less Flow rate of 0.9 mL/min	453012	4.830	5687	1.6
More Flow rate of 1.1 mL/min	398654	3.979	5602	1.5
Less organic phase	445983	3.266	5643	1.55
More organic phase	402315	2.147	5699	1.51

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Aspirin and Prasugrel in bulk drug and pharmaceutical dosage forms.

This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps.

Aspirin and Prasugrel was freely soluble in ethanol, methanol and sparingly soluble in water.

Acetonitrile: water (35:65% v/v) was chosen as the mobile phase. The solvent system used in this method was economical.

The %RSD values were within 2 and the method was found to be precise.

The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.

This method can be used for the routine determination of Aspirin and Prasugrel in bulk drug and in Pharmaceutical dosage forms.

ACKNOWLEDGEMENT

The Authors are thankful to the Management and Principal, Department of Pharmacy, Pydah College of Pharmacy , Kakinada, Andhra Pradesh, for extending support to carry out the research work. Finally, the authors express their gratitude to the Sura Labs, Dilsukhnagar, Hyderabad, for providing research equipment and facilities.

REFERENCES

1. Dr. Kealey, Haines PJ. Analytical chemistry. 1st ed. Bios Publisher; 2002. P. 1-7.
2. BraithWait A, Smith FJ. Chromatographic methods. 5th ed. Kluwer Academic Publishers; 1996. P. 1-2.
3. Weston A, Phyllisr. Brown, HPLC principle and practice. 1st ed. Academic press; 1997. P. 24-37.
4. Kazakevich Y, Lobrutto R. HPLC for pharmaceutical scientists. 1^{s} ed. Wiley Interscience A JohnWiley & Sons, Inc Publishing House; 2007. P. 15-23.
5. Chromatography [online]. Wikipedia. Available from: http://en.wikipedia.org/wiki/Chromatography.
6. Meyer VR. Practical high-performance liquid chromatography. 4th ed. England: John Wiley & Sons Ltd; 2004. P. 7-8.
7. Sahajwalla CG a new drug development. Vol.141. New York: Marcel Dekker, Inc; 2004. P. 421-6.
8. Introduction to column [online]. Available from: http://amitpatel745.topcities.com/index_files/study/columncare.pdf.
9. Detectors used in HPLC (online). Available from: http://wiki.answers.com/Q/What_detectors_are_used_in_HPLC.
10. Detectors [online]. Available from: http://hplc.chem.shu.edu/NEW/HPLC_Book/Detectors/det_uvda.html.
11. Detectors [online]. Available from: http://www.dionex.com/enus/webdocs/64842-31644-02_PDA-100.pdf.
12. Detectors [online]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8867705.
13. Detectors [online]. Available from: http://www.chem.agilent.com/Library/applications/59643559.pdf.
14. Detectors [online]. Available from: http://hplc.chem.shu.edu/new/hplcbook/detector.
15. Draft ICH. Guidelines on Validation of Analytical Procedures Definitions and terminology. Fed Regist. 1995;60:1126.