Journal of Applied Pharmaceutical Science Vol. 3 (08), pp. 006-015, August, 2013
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2013.3802
ISSN 2231-3354 (cc) BY-NC-SA

Development and Validation of RP-HPLC Method for the Determination of Related Compounds in Quetiapine Hemifumarate Raw Material and Tablets

Paulo Cesar Pires Rosa[1]*, Iury Fernando Rodrigues Pires[2], Blanca Elena Ortega Markman[3], Fábio Ferreira Perazzo[1]
[1]Universidade Federal de São Paulo – Instituto de Ciências Ambientais, Químicas e Farmacêuticas, Laboratório de Insumos Naturais e Sintéticos, Campus Diadema-Unifesp; [2]Universidade Federal de Goiás – Instituto de Patologia Tropical e Saúde Pública, Laboratório de Microbiologia Médica, Ambiental e Biotecnologia, Campus Goiânia-UFG; [3]Núcleo de Ensaios Físicos e Químicos em Medicamentos, Centro de Medicamentos, Cosméticos e Saneantes. Instituto Adolfo Lutz, São Paulo, 04021-001, Brazil.

ARTICLE INFO

Article history:
Received on: 11/07/2013
Accepted on: 05/08/2013
Available online: 30/08/2013

Key words:
Quetiapine hemifumarate, high performance liquid chromatographic, related compounds, validation.

ABSTRACT

A simple and sensitive high performance liquid chromatographic method has been developed for the determination of assay quantitative of related compounds and quetiapine hemifumarate in raw material and tablets. Quetiapine hemifumarate is used for the treatment of schizophrenia and there are some generic medicines available in brazilian marketing pharmaceutical, it´s necessary evaluate the quality control of raw material used in the production. Efficient chromatographic separation was carry out on a C18 stationary phase with mobile phase consisting in a mixture of phosphate buffer pH 6.6:Acetonitrile:Methanol (45:40:15), flow rate of 1.0 mL min^{-1}, injection volume of 20 µL, temperature of 25 °C and ultraviolet detection at 220 nm. All of the chromatographic parameters were attended, with resolution greater than 2.9 between quetiapine hemifumarate and impurities. The HPLC method was validated according ICH guidelines, evaluating selectivity, limits of detection and quantification, linearity, accuracy, precision and robustness. The relative retentions times were about 0.58, 0.69 and 0.88 to related compounds, piperazine, lactam and ethanol compound, respectively. Impurities were found < 0.1 % in samples and the assay of quetiapine hemifumarate was > 98.15%. The method can be used for the routine analysis of the impurities in Quetiapine hemihumarate (QH) without any interference.

INTRODUCTION

Quetiapine hemifumarate, 1-[2-(2-hydroxyethoxy) ethyl] -4-(dibenzo [b,f][1,4] thiazepin-11-yl) piperazinium hemifumarate, (Figure 1) is an atypical antipsychotic drug, belong to the dibenzothiazepine derivatives. Quetiapine is prescribed for the treatment of schizophrenia and can be used for the treatment of acute manic episodes associated with bipolar (Peuskens *et al*., 2007, Cutler *et al*., 2008).

At Brazil there is a commercial formulation Seroquel and, at least, 12 generic medicine of quetiapine hemifumarate approved for the regulatory agency (ANVISA 2013a). Quetiapine hemifumarate is the first in this new class of antipsychotic medications, especially for patients who have not benefited from the classical antipsychotics.

QH is not official in most important pharmacopeias used in Brazil, BP (Brasiliam Pharmacopeia), BP (British Pharmacopoeia 2013), EP (The European Pharmacopoeia British Pharmacopoeia 2013, 7ª Edition 7.7) and USP (The United States Pharmacopoeia 35). An available reference is in the Pharmacopeial Forum 37(3), but in a review process. In this monography, there is an impurities' analysis description, but not official yet. Other reference is to dissolution methods for tablets of QH founded in FDA.

QH has some related compounds that are evaluated both in the raw and in the tablet. The analytical method for this application must have adequate selectivity for the analyte of interest present in samples containing similar compounds. Among the available methods, HPLC is a separation technique based in establish principles of liquid chromatography, which that allows separation of compounds of interest in complex matrices and has found time most important test used in recent years in pharmaceuticals and biomedical analysis.

* *Corresponding Author*
Paulo Cesar Pires Rosa, Laboratório de Insumos Naturais e Sintéticos, Campus Diadema-Unifesp, São Paulo, 04021-001, Brazil.
Phone:. +55-1133193578

© 2013 Paulo Cesar Pires Rosa et al. This is an open access article distributed under the terms of the Creative Commons Attribution License -NonCommercial-ShareAlike Unported License (http://creativecommons.org/licenses/by-nc-sa/3.0/).

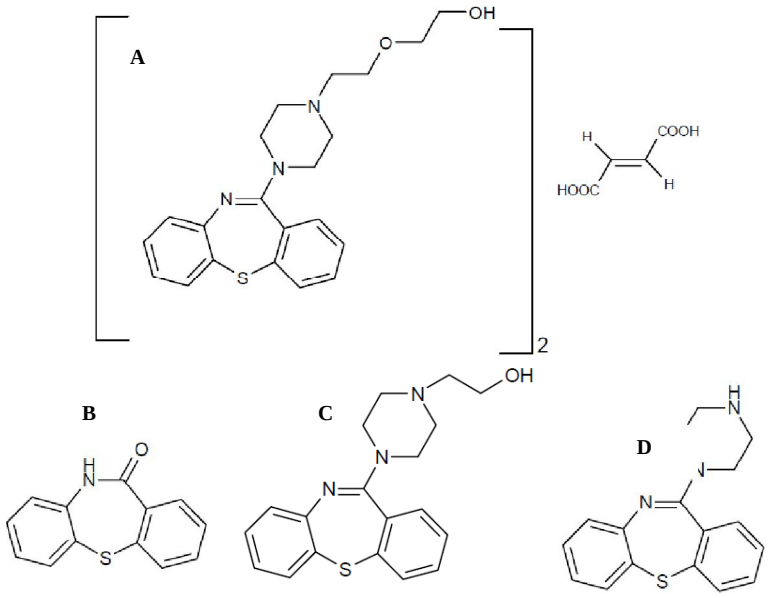

Fig. 1: Chemical structure of quetiapine hemifumarate (A) and related compounds lactam compound (B), ethanol compound (C) and piperazine compound (D).

A review of literature for quetiapine shows that QH has been determined in drug substance, pharmaceuticals formulations and biological matrices for analytical techniques, but few analytical methods are available using HPLC, to determine related compounds including: polarographic determination for the analysis of quetiapine in pharmaceuticals (Vinay *et. al*., 2010), analysis with electrochemical sensor (Vigovic *et al*., 2001), voltammetric analysis in human serum and urine (Ozkan *et al*., 2006), polarographic analysis of quetiapine in pharmaceuticals (El-Enany *et al*., 2009), with other atypical antipsychotics by capillary zone electrophoretic method (Hillaert *et al*., 2004), by HPTLC in pharmaceutical dosage form (Dhaneshwar *et al*., 2009), comparative study of a spectrophotometric method and a capillary zone electrophoretic method (Pucci *et al*., 2003), GC–FID determination of non-chromophoric impurities (Jadhav *et al*., 2010).

QH have been determined in humam plasma by LC–MS/MS (Mandrioli *et al*., 2002; Barret *et al*., 2007; Davis *et al*., 2010; Xiong *et al*., 2013; Ansermot *et al*., 2013) and in oral fluid by LC–MS/MS (Di Corcia *et al*., 2013). The analysis of QH by HPLC with UV detection has been carry out in tablets and human plasma (Belal *et al*., 2008) and stability indicating HPLC method for the determination of related substances in quetiapine fumarate have also published (Radha *et al*., 2008; Soma *et al*., 2009; Trivedi *et al*, 2011).

The presence of related compounds has been an important evaluation being carried out by regulatory agencies. As yet a monograph recognized for determination of impurities in QH is important to use an analytical method that separates and identify impurities in raw materials and tablets. In the present work, a reversed phase – HPLC Method has been applied to the method development and validation study of assay determination of quetiapine hemifumarate and related compounds in raw material and in dosage forms.

MATERIAL AND METHOD

Instruments and Apparatus

RP-HPLC instrumentation Waters (Massachusetts – USA) consisting of management module solvents and samples Alliance 2695, Photodiode Array Detector 2998. Data acquisition was performed using the chromatography software package Empower 3.0. For Robustness study was used a HPLC system Shimadzu (Japan) consisting of a quaternary pump (L-7130), degasser, column oven (L-2300) and automatic injector (L-2200). Quantification and peak purity were checked with a photodiode array detector (L-2450). Data acquisition was performed using the chromatography software package LC – Solution. The chromatographic separations were performed using a C18 column (250 mm x 4.6 mm id, 3 µm particle size). Analytical Balance (Satorius, Germany), Ultrasonic bath Unique (Brazil), Corning volumetric flasks, pipettes of borosilicate glass were used in study, Water Purification System (Millipore Bioscience Division) and pH meter model 744 Metrohm (Herisau-Switzerland) was used during study.

Chemicals and Reagents

Quetiapine fumarate and impurities: Impurity Lactam Compound - Dibenzo[b,f][1,4]thiazepin-11(10H)-One; Impurity Piperazine Compound: 11-Piperazino-dibenzo[b,f][1,4]thiazepine dihydrochloride; Impurity Ethanol Compound: 11-[4-(2-Hydroxyethyl)] -1- piperazinyl]- dibenzo [b,f] [1,4]thiazepine) reference standards (MOEHS Productos Quimicos, Spain), were used without further purification. Tablet preparations containing 200 mg Quetiapine Hemifumarate were purchase from brazilian marketing pharmaceutical. Potassium dihydrogen phosphate was obtained from J. T. Baker (Eldorado de Mexico, Mexico). HPLC grade acetonitrile and methanol were purchased from J.T. Baker (Phillipsburg, USA). Water was deionized and purified by means

of a Milipore MilliQ and filtered through a 0.45 µm nylon filter obtained from Millipore.

Chromatographic Conditions

The injection volume to carry out the chromatography was set at 20 µL. The chromatographic parameters: retention factor (k), plate number (N), resolution (RS) and tailing factor (TF) were calculated to evaluate the separation. The detector wavelengths were at 220 nm.

The mobile phase used in this study consisted of a mixture of acetonitrile:methanol:phosphate buffer (pH 6.6) at a proportion of 40:15:45 volume to volume, in the isocratic mode with effluent flow rate monitored at 1.0 mL min^{-1}. The pH 6.6 phosphate buffer solution was prepared by added 1.2 mL of phosphoric acid in 1000 mL of the water purified and pH adjusted to 6.6 with addition of triethylamine. The solvents and buffer were filtered through a 0.45 µm GH-membrane filter and degassed ultrasonically prior to use. The temperature of the column was held at 25 °C. A proportion of sample and standard solutions were taken and filtered through a PVDF membrane filter (0.45 µm), before injection.

Preparation of Solutions

Standard stock solutions of QH and impurities (125 µg mL^{-1})

A standard stock solution of quetiapine hemifumarate and each impurity was prepared individually by accurately weighing 6.25 mg of the standard of impurity, dissolving this in mobile phase and diluting to 50 mL in a volumetric flask (125 µg mL^{-1}).

Working solutions

A 1.0 mL volume of each stock solution prepared was transferred to 100 mL volumetric flasks. The working solutions of each compound were diluted to volume with mobile phase to obtain 1.25 µg mL^{-1}.

Mixed standard solution of QH and impurities

A 1.0 mL volume of each stock solution prepared was transferred to the same 100 mL volumetric flasks. The solution was diluted to volume with mobile phase to obtain 1.25 µgmL^{-1}. A proportion was taken and filtered through a PVDF membrane filter (0.45 µm), before injection.

Sample solutions

A weight equivalent of 25.0 mg of QH of the raw material or tablets was transferred to a 100 mL volumetric flask, 70.0 mL of mobile phase was added and the solution was stirred until dissolution. After that the solution was diluted to volume with mobile phase. Appropriate proportions were taken and filtered through a PVDF membrane filter (0.45 µm) and used for the determination of impurities. Each of the solutions prepared were injected in triplicate into the chromatographic system. Concentration of quetiapine hemifumarate: 250 µgmL^{-1}.

Sample solution dopped

A weight of 25.0 mg of raw material was transferred to a 100 mL volumetric flask, 70.0 mL of mobile phase was added and the solution was stirred until dissolution. A 1.0 mL volume of each impurity stock solution was added. After that the solution was diluted to volume with mobile phase. Appropriate proportions were taken and filtered through a PVDF membrane filter (0.45 µm) and used for the determination of impurities. Concentration of quetiapine hemifumarate: 250 µgmL^{-1}, Concentrations of impurities: 1.25 µgmL^{-1}

Validation of method

Validation of an analytical method must demonstrate that it fulfill all the requirements of the analytical method application, ensuring the reliability of the results. The validation was carry out by studying the parameters specificity, linearity, precision, sensitivity, accuracy, solution stability and limits of detection and quantification adequate for the analysis (ANVISA, 2003; ICH, 1995A; ICH, 1995B; INMETRO 2007, Rosa *et al*., 2012). Solutions were filtered through a PVDF membrane filter (0.45 µm), before injection.

System suitability

For system suitability studies, five replicate injections of the mixed standard solution were made, and parameters such as relative standard deviation (RSD) of the peak area, capacity factor, column efficiency, resolution and the tailing factor of the peaks were calculated.

Specificity

The specificity of the method was investigated by analyzing chromatograms obtained from the placebo, mobile phase, standard and sample solutions and comparing the retention times of components of the samples to be analyzed with standards of QH and impurities. The peak purity of QH and impurities in the sample and in the standard preparation was also verified by comparisons of the spectra obtained with the diode array detector.

Detection limit (LOD) and quantitation limit (LOQ)

The LOD and LOQ were determined based on signal-to-noise ratios of the analytical responses at 3 and 10 times the background noise, respectively. Solutions of standard of QH and impurities were prepared in the range of 1 – 10% of the target level concentration. To solution of 1%, a 10 µL volume of each stock solution prepared was transferred to 100 mL volumetric flasks. The quantification limit solutions were diluted to volume with mobile phase to obtain 0.0125 µgmL^{-1}. Other solutions contending 0.025, 0.050, 0.100 and 0.125 µgmL^{-1} were prepared to evaluate LOD and LOQ.

Calibration and linearity

The linearity of the method was tested in the range of LOQ-120% of the target level of the assay concentration of QH and impurities (1.25 µg mL^{-1}). Aliquots of the standard stock solutions of QH and impurities were taken in different volumetric flasks and diluted with mobile phase to obtain final concentrations

in the range, LOQ and 0.50 μgmL^{-1} to 1.50 μgmL^{-1} of each compound. A 400 µL volume of the each stock solution prepared was transferred to 100 mL volumetric flasks. The standard solutions were diluted to volume with mobile phase to obtain 0.50 μgmL^{-1}.

A proportion was taken and filtered through a PVDF membrane filter (0.45 µm), before injection. Other calibrations solutions were prepared transferred 600, 800, 1000 and 1200 µL of stock solution to 100 mL volumetric flasks to obtain concentrations of 0.75, 1.00, 1.25 and 1.50 μgmL^{-1}, respectively. Each of the levels of concentration was injected in triplicate. Peak area versus concentration data were performed by linear least-squares regression analysis and the analytical curves were found to be linear for all compounds in the mentioned concentrations.

Precision

The precision was evaluated by intraday (repeatability) and interday (intermediate precision) studies. The intraday precision was investigated using six different sample solutions prepared from sample of raw material and tablets of QH with and without impurities at 100% of the target level for both the impurities.

Each solution was injected in triplicate and the peak areas obtained were used to calculate mean and RSD values of each compound. The interday precision was checked by preparing, and analyzing in triplicate, six different sample solutions from the samples at the same concentration levels as used for intraday precision, on different days, by different analysts and/or different HPLC systems (ANVISA, 2003). The percent of the assay for the two components was calculated using the analytical curve.

Accuracy

The accuracy of the method was evaluated by recovery experiments. The recovery experiments were performed by fortifying the sample of raw material and tablets with three known concentrations of the impurities, at the levels of LQ, 100% and 120% of the target concentrations (1.25 μgmL^{-1}). Three samples were prepared for each recovery level. The amount of impurities recovered was calculated in relation to the added amount. Each solution was injected in triplicate and peak areas were used to calculate the mean and % RSD values.

Robustness

A study of robustness was carried out by testing the susceptibility of the measurements to deliberate variations of the analytical conditions in the response of samples and standards solutions. The factors chosen for this study were the temperature (°C), pH, flow rate (mL min^{-1}), mobile phase (% of acetonitrile), different columns bath and different chromatographic system.

Solution stability

To determine the stability of the standard and sample solutions of QH and impurities, the assay method was carried out after leaving the test solutions of both sample and reference in tightly capped volumetric flasks at room temperature for 72 h. The sample solutions were assayed at 18 h intervals throughout the study period. Mobile phase stability was also analyzed by assaying the freshly prepared sample solutions against freshly prepared reference standard solutions at 18 h intervals up to 72 h. Mobile phase was kept constant during the study period.

RESULTS AND DISCUSSION

Optimization of the chromatographic conditions.

The developed HPLC method for determination of related compounds in pharmaceuticals must present chromatographic parameters resolution, capacity factor, plate number and tailing factor according as described by the American pharmacopoeia (USP 2012). In this sense, the chromatographic conditions such as composition and flow rate of mobile phase, stationary phase type, particle size, column dimensions, column temperature, sample concentration and injection volume should be evaluated so that the method presents the minimum acceptance. The method should be able to determine assay and should be accurate, reproducible, robust, free of interference from blank, placebo, impurities and straightforward enough for routine use in quality control laboratory. To optimize the RP-HPLC parameters, several mobile phase compositions were tried. Evaluation of the influence of buffer pH was one of the main factors in separating all the impurities in quetiapine hemifumarate. When the pH of the buffer (sodium dihydrogen phosphate) was adjusted to 6.6 the ionization of related compounds increased, subsequently the interaction with the column decreased, reducing retention time e obtaining better separation. At the same time, to optimize the retention time of quetiapine hemifumarate and resolution, different composition were tried and a mobile phase with a higher percentage of acetonitrile was selected to reduce retention time. The mobile phase consisting acetonitrile:methanol:phosphate buffer (pH 6.6) at a proportion of 40:15:45 volume to volume, in the isocratic mode with effluent flow rate monitored at 1.0 mL min^{-1}, was found to be satisfactory to obtain good peak symmetry, resolution, better reproducibility and repeatability for QH and impurities. To further improve the retention time, resolution and symmetry of the related compounds and quetiapine hemifumarate peaks, column length and particle size were studied. Thus, the column used was a C18 column 250 x 4,6 mm, 3 µm particle size.

Validation of Method

System suitability and Specificity

Complete resolution of the peaks with clear baseline was obtained, Figure 2 to 4. The retention times and system suitability test parameters for QH and impurities for the proposed method are reported in Tables 1 to 3, for standard, sample doped with impurities and sample as is chromatograms, respectively. Peak purity of QH and impurities were confirmed by comparing the spectra of standard and sample solutions using the software Empower, one example for sample solution is showed in Figure 5, showing that doesn´t have interference of other compounds,

mobile phase and formulation excipients. The relative retention time (RRT) was about 0.57 for Piperazine Compound, 0.71 for Lactam Compound, 0.87 for Ethanol Compound and 1.0 for QH. The results obtained for system suitability are according with general chapter chromatography of the American Pharmacopoeia (USP 35, 2012).

Calibration and linearity

The response was found linear for QH and impurities from LOQ (5%) to 120 % of standard concentration and correlation coefficient was also found greater than 0.99. The regression analysis and linearity range data for the proposed method, LOD and LOQ were calculated and are in Table 3.

The LOD and LOQ obtained were equivalent to 1% and 4% of the target concentrations (1.25 μgmL^{-1}). Response factor was calculated by ratio between slope of impurity and QH, showing similar value for impurities. Figure 6 shows a comparative graphic of the calibration curve of the QH and impurities for proposed method. The evaluation chart residues showed no trend deviations for results as showing in Figure 7.

Precision

The assay results and % RSD, calculated from the peak areas for impurities and QH are shown in Tables 4 and 5, indicate that the method is reliable. Value of F test indicates that there were not statistically significant differences between precision intermediate.

Accuracy

To confirm the accuracy of the proposed method, recovery experiments were carried out by standard impurities addition of technique.

The mean of percentage recoveries for each level and the % RSD was calculated. The recovery values for amount of impurities from 96.79 % to 101.66 %. Results indicate that the method is accurate and also there is no interference due to other impurities present in raw material. The average recovery of the three levels (nine determinations) is shown in Table 6.

Robustness

In the study of robustness was measure of method capacity to remain unaffected by small, but deliberate changes in chromatographic conditions. Between parameters studied no significant effect was observed on system suitability parameters such as resolution, theoretical plates, tailing factor and capacity factor. The results of each impurity were available by ratio of area between of normal condition and changed condition. The chromatographic conditions changed and results are presented in Table 7. All the results are conform specification of the 95% to 105% of recovery.

The method was found to be robust with respect to variability of the conditions studied. To exemplify the use of the method, a comparison with a different column C18, 250 x 4.6 mm i.d., 5 μm particle size, and different chromatographic system with photodiode arranje as detector was carried out. The results obtained from analysis with the other column and chromatographic system was similar to the chromatographic separation from the original condition. Figure 8 shows a chromatogram of standard solution of QH and impurities with different column and chromatographic system in the study of robustness. The system suitability obtained with different conditions is shown in the Table 8.

Stability of solution

Stability of sample and standard solutions were evaluated by determination of the % RSD for the assay values determined up to 72 h for the sample preparation doped with impurities. The assay values were within 2 % after 72 hours. The results indicate that the solutions were stable for this time when stored at ambient temperature.

Sample and Standard solutions did not show any appreciable change in assay value when stored at ambient temperature.

Analysis of sample of raw material and tablets

The method was applied for the analysis of two raw material of QH available and five marketed formulations containing 200 mg of QH per tablet.

The results of analysis of raw material and tablet formulations are shown in Table 9. All of them meet requirement of few 0.15 % of the related compounds described at the Pharmacopeial Forum (USP Pharmacopeial Forum, 2013).

CONCLUSION

A simple RP-HPLC method was successfully developed for the determination of related compounds and quetiapine hemifumarate in raw material and tablets. The method was validated and considered selective with resolution greater than 2.9 between quetiapine hemifumarate and impurities, linear with coefficient of correlation, (r^2) above 0.99 for all compounds studied, precise with % RSD adequate for the precision study, robust for all chromatographic variations and accurate with recovery of 96% to 102%.

The result of the analysis impurities of raw material by the proposed method was highly reproducible and reliable and it was in good agreement with the label claim of the drug. The method can be used for the routine analysis of the impurities in QH without any interference.

ACKNOWLEDGMENTS

The authors acknowledge financial support from the Brazilian Agencies FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico).

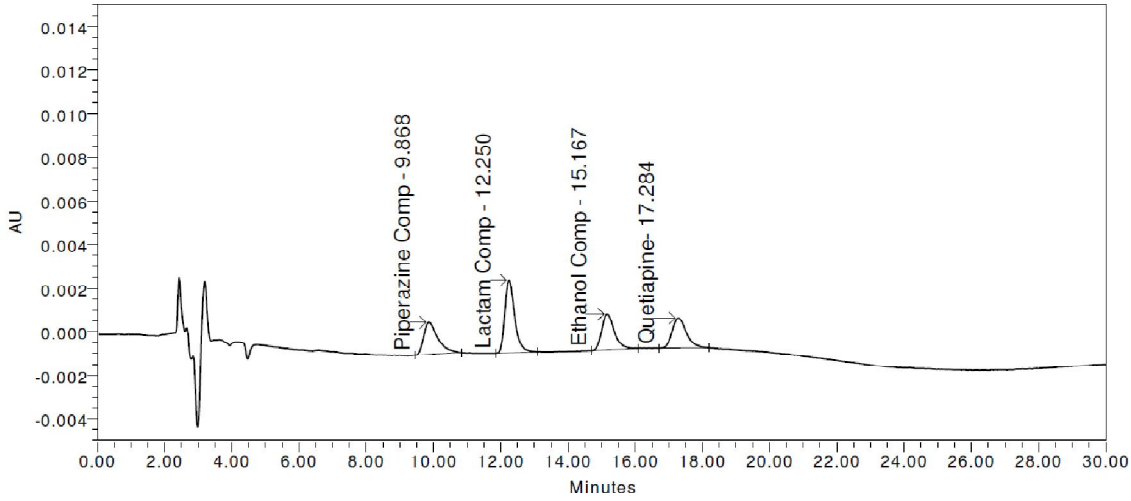

Fig. 2: Chromatogram of standard solution of quetiapine hemifumarate and impurities.

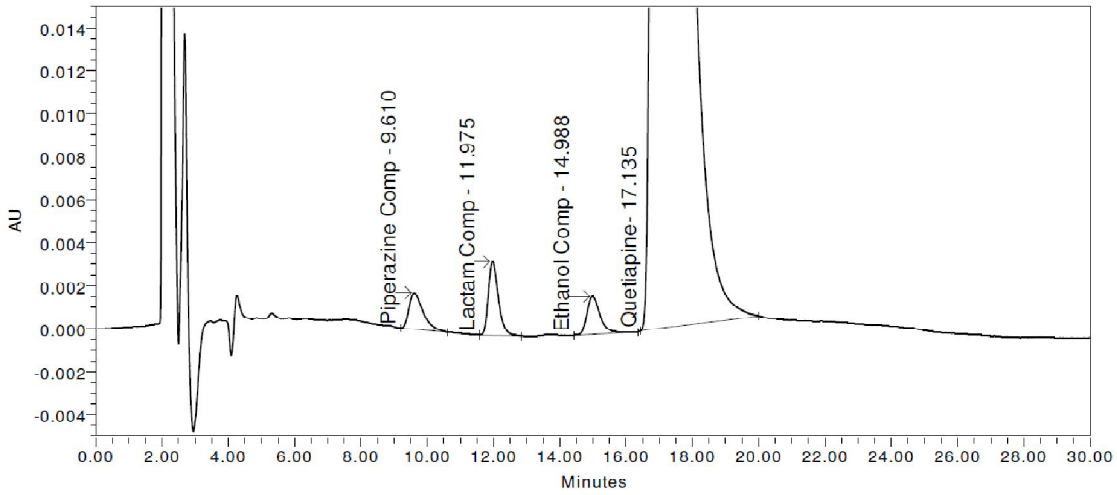

Fig. 3: Chromatogram of sample solution of quetiapine hemifumarate doped with impurities.

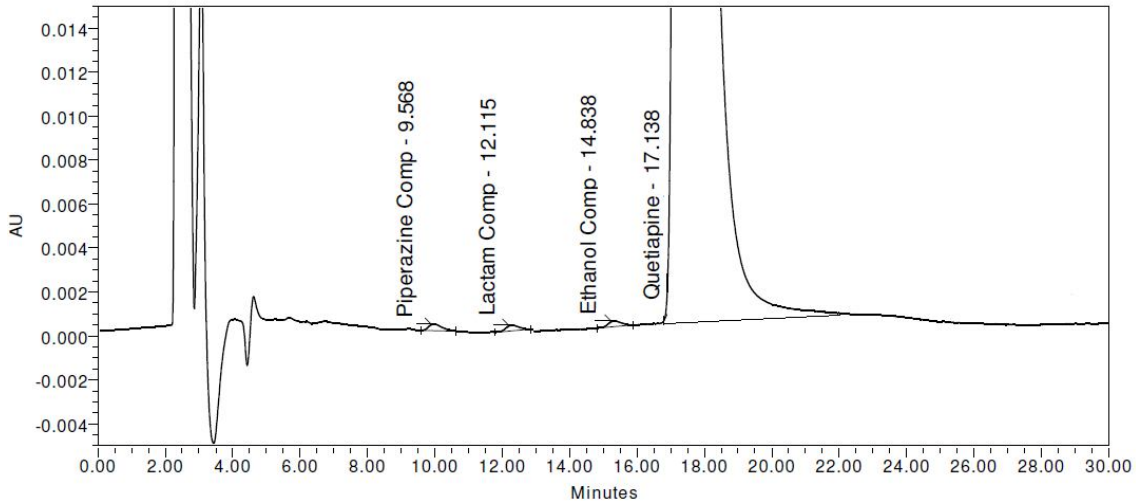

Fig. 4: Chromatogram of sample solution raw material of the quetiapine hemifumarate.

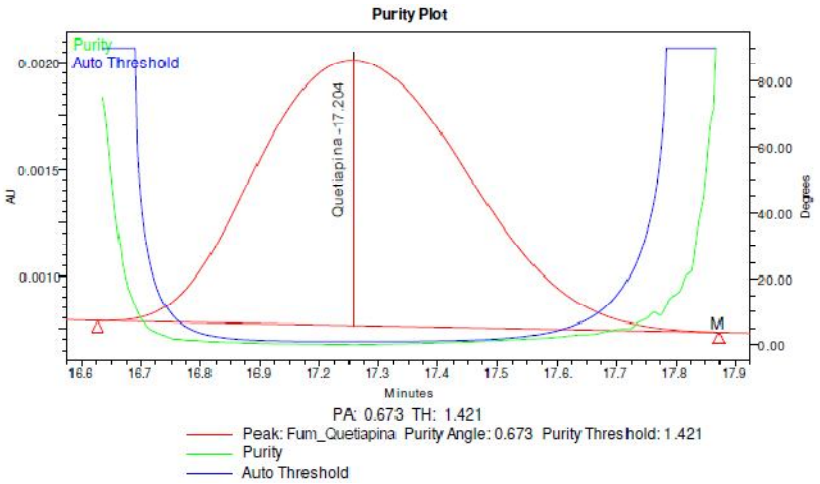

Fig. 5: Peak purity of quetiapine hemifumarate in standard solution.

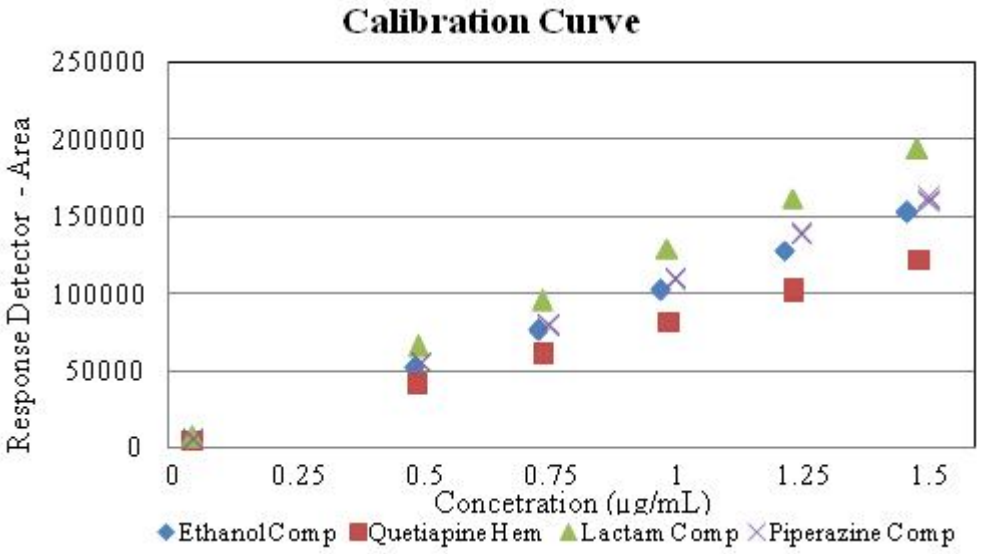

Fig. 6: Calibration curve of the quetiapine hemifumarate and impurities for proposed method.

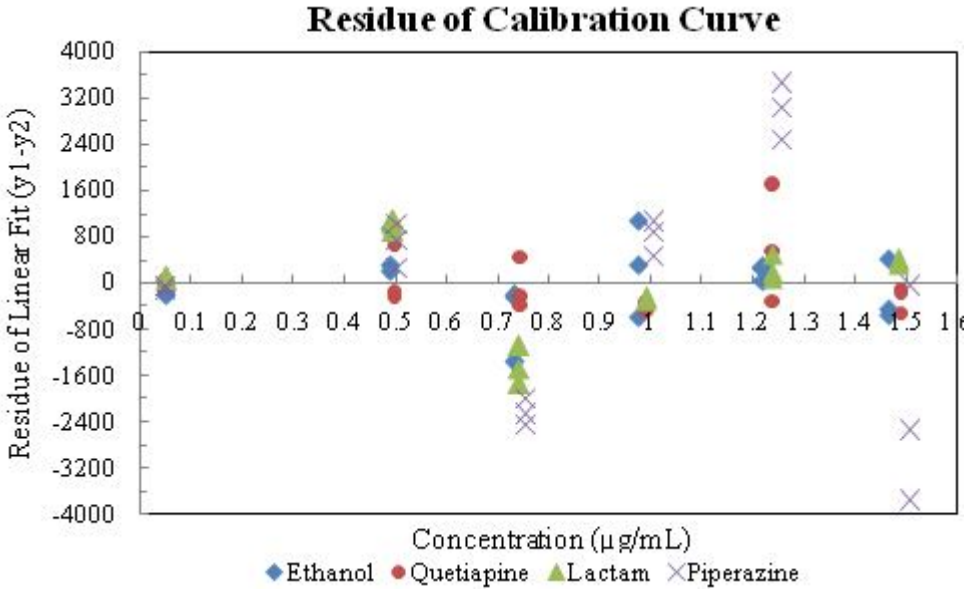

Fig. 7: Residue of the calibration curve for proposed method.

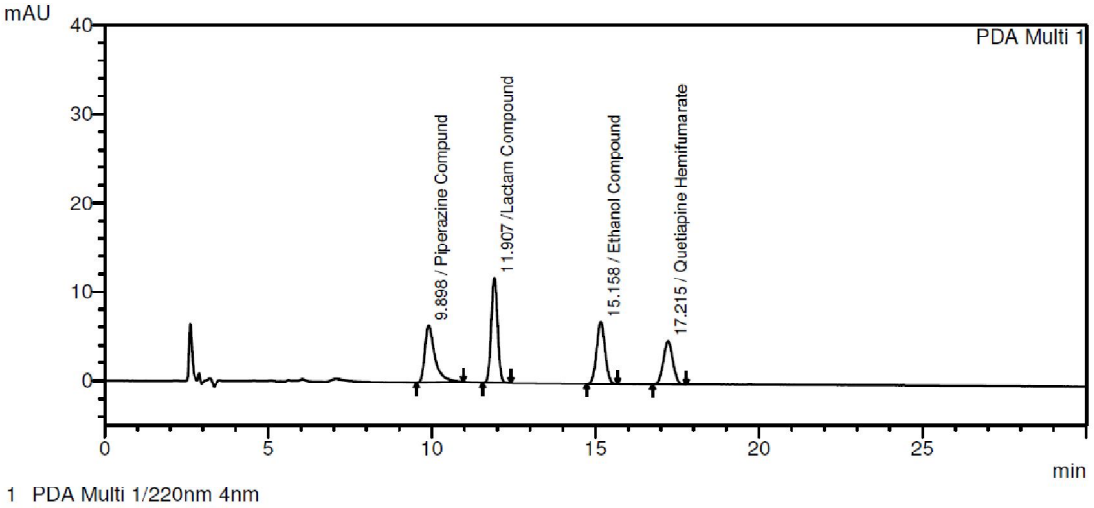

Fig. 8: Chromatogram of standard solution of quetiapine hemifumarate and impurities with different column and chromatographic system in the study of robustness.

Table. 1: System suitability parameters of standard chromatogram obtained to QH and impurities.

Parameters	HPLC Method			
	Piperazine Compound	Lactam Compound	Ethanol Compound	Quetiapine Hemifumarate
Retention time	9.87	12.25	15.17	17.28
Capacity factor	3.74	4.89	6.29	7.31
Taling factor	1.63	1.35	1.30	1.32
Plates number	2623	8965	8255	7999
Resolution	-----	3.66	4.82	2.91

Table. 2: System suitability parameters of sample chromatogram obtained to QH and impurities.

Parameters	HPLC Method			
	Piperazine Compound	Lactam Compound	Ethanol Compound	Quetiapine Hemifumarate
Retention time	9.61	11.98	14.99	17.14
Capacity factor	3.62	4.76	6.21	7.24
Taling factor	1.73	1.38	1.23	1.38
Plates number	2465	7889	7314	7630
Resolution	-----	3.51	4.78	2.83

Table. 3: Regression analysis data for the proposed method.

Parameters	Compounds HPLC Method			
	Piperazine Compound	Lactam Compound	Ethanol Compound	Quetiapine Hemifumarate
Range (μgmL^{-1})	0.0503-1.5087	0.0495-1.4843	0.0488-1.4641	0.0496 -1.4880
Correlation coeficient	0.9986	0.9998	0.9999	0.9998
Slope	107788	129974	104254	81369
Intercept	- 381,1	129,8	20,8	69,4
LOD (μgmL^{-1})	0.0126	0.0125	0.0127	0.0126
LOQ (μgmL^{-1})	0.0503	0.0495	0.0488	0.0496
Sinal/noise	10.33	15.76	13.64	11.21
Response factor	0.7549	0.6260	0.7805	1.0000

Table. 4: Precision data for proposed method for sample as is.

Amount Founded % /Precision	Recovery HPLC Method		
	Piperazine Compound	Lactam Compound	Ethanol Compound
Repeatability %	0.0380	0.0154	0.0491
%RSD	3.20	2.09	2.09
Intermediate %	0.0361	0.0145	0.0470
%RSD	2.10	2.90	3.44
F test*	2.57	1.70	2.48

* F teste value 5.05 for confiance level 95%, six samples.

Table. 5: Precision data for proposed method for sample doped with impurities.

Amount Founded % /Precision	Recovery HPLC Method		
	Piperazine Compound	Lactam Compound	Ethanol Compound
Repeatability %	0.5422	0.05060	0.5380
%RSD	1.74	1.69	1.65
Intermediate %	0.5357	0.5009	0.5322
%RSD	1.99	1.55	1.53
F test*	1.28	1.21	1.19

Table. 6: Recovery data obtained in study of accuracy for proposed method.

Fortification Level		Recovery HPLC Method			
		Piperazine Compound	Lactam Compound	Ethanol Compound	Quetiapine Hemifumarate
LOQ	Amount %	96.79	97.37	96.32	98.11
	% RSD	0.81	1.29	1.36	1.79
100%	Amount %	101.66	98.58	97.65	99.50
		0.54	1.13	0.66	1.46
120%	Amount %	99.48	97.81	99.60	99.67
		0.32	0.20	0.52	0.69

% RSD: relative standard deviation of three samples.

Table. 7: Results of recovery data for robustness for proposed method.

Fortification Level	Variation	Recovery			
		Piperazine Compound	Lactam Compound	Ethanol Compound	Quetiapine Hemifumarate
Temperature (°C)	28	99.13	100.87	100.60	100.31
	32	99.27	100.41	99.87	100.59
Flow rate	0.95	99.89	100.07	100.56	100.31
	1.05	102.21	100.68	99.28	96.48
Amount acetonitrile %	37	99.23	101.06	100.75	100.14
	43	99.27	100.70	100.43	100.56
pH	6.4	103.91	100.16	100.74	98.37
	6.8	101.79	97.34	98.68	99.56
Column Dimension	25 cm x 3,9 mm, 5µm	102.48	99.84	98.47	101.01
Chromatographic System	Shimadzu DAD	101.15	100.47	99.38	100.29

Table. 8: System suitability parameters of standard chromatogram obtained in robustness study with different chromatographic system.

Parameters	HPLC Method - Robustness			
	Piperazine Compound	Lactam Compound	Ethanol Compound	Quetiapine Hemifumarate
Retention time	9.90	11.91	15.16	17.22
Capacity factor	3.76	4.73	6.29	7.28
Taling factor	1.56	1.07	1.05	1.04
Plates number	5721	17078	15915	16362
Resolution	-----	4.53	7.70	4.04

Table. 9: Analysis of raw material and marketed formulation of QH by proposed method (n = 3).

Sample	% Founded of QH and Related Compounds			
	Piperazine Compound	Lactam Compound	Ethanol Compound	Quetiapine Hemifumarate*
Raw material 1	0.03	0.04	0.02	99.85 ± 0.51
Raw material 1	0.04	0.05	0.01	99.91 ± 0.54
Tablet 1	0.02	0.03	0.02	98.54 ± 0.48
Tablet 2	0.05	0.06	0.04	99.05 ± 0.42
Tablet 3	0.04	0.05	0.05	98.15 ± 0.55
Tablet 4	0.08	0.09	0.07	99.37 ± 0.34
Tablet 5	0.07	0.08	0.05	98.71± 0.39

* Amount found about drug declared ± standard deviation.

REFERENCES

ANVISA - AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA. RE nº 899 de 29 de maio de 2003. Guia para validação de métodos analíticos e bioanalíticos. Diário Oficial da União Poder Executivo, Brasilia, 02 jun. 2003. Available at: http://portal.anvisa.gov.br Acessed on: 10 may 2013.

ANVISA – Agencia Nacional de Vigilância Sanitária, 2013a http://portal.anvisa.gov.br/wps/portal/anvisa/anvisa/home/medicamentos.

Ansermot N., Brawand-Amey M., Kottelat A., Eap CB., Fast quantification of ten psychotropic drugs and metabolites in human plasma by ultra-high performance liquid chromatography tandem mass spectrometry for therapeutic drug monitoring. J. Chromatogr. A., 2013, 1292: 160– 172.

Barrett B., Holcapek M., Huclová J., Borek-Dohalský V., Fejt P., Nemec B., Jelínek I., Validated HPLC–MS/MS method for determination of quetiapine in human plasma. J. Pharm. Biomed. Anal., 2007, 44: 498–505.

Belal F., Elbrashy A., Eid M., Nasr JJ. Stability-Indicating HPLC method for the determination of quetiapine: Application to tablets and human plasma. J Liq Chrom Rel Technol. 2008; 31: 1283–1298.

Cutler A., Earley W., Datto D., Effectiveness of the extended-release formulation of quetiapine as monotherapy for the treatment of acute bipolar mania. Int. J. Neuropsychopharmacol, 2008, 11:184–5.

Davis PC., Bravo O., Gehrke M., Azumaya CT., Development and validation of an LC–MS/MS method for the determination of quetiapine and four related metabolites in human plasma. J. Pharm. Biomed. Anal., 2010, 51: 1113–1119.

Di Corcia D., Lisi S., Pirro V., Gerace E., Salomone A., Vincenti M., Determination of pharmaceutical and illicit drugs in oral fluid by Ultra-high performance liquid chromatography–tandem mass spectrometry. J. Chromatogr. B, 2013, 927; 133– 141.

Dhaneshwar SR., Patre NG., Mahadik MV., Stability-indicating HPTLC method for quantitation of quetiapine fumarate in the pharmaceutical dosage form. Acta Chromatogr. 2009, 21: 83–93.

El-Enany N., El-Brashy A., Belal F, El-Bahay N. Polarographic analysis of quetiapine in pharmaceuticals. Port Electrochim Acta, 2009, 27: 113–125.

Hillaert S., Snoeck L., Van den Bossche W., Optimization and validation of a capillary zone electrophoretic method for the simultaneous analysis of four atypical antipsychotics. J Chromatogr A. 2004, 1033: 357–362.

INMETRO - INSTITUTO NACIONAL DE METROLOGIA. Qualidade e Tecnologia. Orientação sobre validação de métodos de ensaios químicos. Rio de Janeiro: INMETRO, 2007. 24 p. (DOQ – CGCRE – 008. Revisão 02).

ICH - INTERNATIONAL CONFERENCE ON HARMONIZATION. Validation of analytical procedures: definitions and terminology. Q2A (CPMP/ICH/381/95). 1995A. Available at: <http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html>. Accessed on: 10 May 2013.

ICH - INTERNATIONAL CONFERENCE ON HARMONIZATION. Validation of analytical procedures: methodology. Q2B (CPMP/ICH/281/95), 1995B. Available at: <http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html>. Accessed on: 10 May 2013.

Jadhav SA., Landge SB., Shelke SS., Niphade NC., Panchangam RK., Mathad VT., Bembalkar SR., Original GC–FID Determination of Non-Chromophoric Impurities in Quetiapine: An Antipsychotic Drug. Chromatographia 2010, 71: 1055-1061.

Mandrioli R., Fanali S., Ferranti A., Raggi MA., HPLC analysis of the novel antipsychotic drug quetiapine in human plasma. J. Pharm. Biomed. Anal., 2002, 30: 969–977.

Nigovic B., Spajic J., A novel electrochemical sensor for assaying of antipsychotic drug quetiapine. Talanta, 2011, 86: 393– 399.

Ozkan SA., Dogan B., Uslu B., Voltammetric analysis of the novel atypical antipsychotic drug quetiapine in human serum and urine. Microchim Acta, 2006, 153: 27–35.

Peuskens J., Trivedi J., Malyarov S., Prevention of schizophrenia relapse with extended release quetiapine fumarate dosed once daily: a randomized, placebo-controlled trial in clinically stable patients. Psychiatry, 2007, 4:34–50.

Pucci V., Mandrioli R., Ferranti A., Furlanetto S., Raggi MA. Quality control of commercial tablets containing the novel antipsychotic quetiapine. J Phar Biom Anal. 2003, 32: 1037–1044.

Radha Krishna S., Rao BM., Someswara Rao N. A validated stability indicating HPLC method for the determination of related substances in quetiapine fumarate. Rasayan J Chem. 2008; 3: 466–474.

Rosa PCP., Jardim ICSF. Simultaneous determination of clobutinol hydrochloride and doxylamine succinate from syrups by RP HPLC using a new stationary phase containing embedded urea polar groups. Braz. J. Pharm. Sci. 2012; vol. 48, no. 2, apr./jun.: 315-323.

Soma Raju IV., Raghuram P., Sriramulu J. Development and Validation of a New Analytical Method for the determination of Related Components in Quetiapine Hemifumarate. Chromatographia 2009, 70: 545-550.

Trivedi RK., Patel MC. Development and Validation of a Stability Indicating RP-UPLC , Sci. Pharm. 2011, 79: 97–111.

UNITED STATES PHARMACOPEIA - USP. USP 35. NF 30. Rockville: United States Pharmacopeia Convention, 2012.

UNITED STATES PHARMACOPEIA – USP, Pharmacopeial Forum 37(3), Rockville: United State, 2013.

Vinay KB., Revanasiddappa HD., Rajendraprasad N., Potentiometric Determination of Quetiapine Fumarate in Pharmaceutical Formulations. Portugaliae Electrochimica Acta, 2010, 28(5), 299-308.

Xiong X., Yang L., Duan J., Development and validation of a sensitive and robust LC–MS/MS with electrospray ionization method for simultaneous quantitation of quetiapine and its active metabolite norquetiapine in human plasma. Clin Chim Acta, 2013, 423: 69–74.

How to cite this article:

Paulo Cesar Pires Rosa, Iury Fernando Rodrigues Pires, Blanca Elena Ortega Markman, Fábio Ferreira Perazzo., Development and Validation of RP-HPLC Method for the Determination of Related Compounds in Quetiapine Hemifumarate Raw Material and Tablets. J App Pharm Sci, 2013; 3 (08): 006-015.