Journal of Chromatography
Separation Techniques

Mevlut et al., J Chromatogr Sep Tech 2018, 9:5
DOI: 10.4172/2157-7064.1000409

Research Article Open Access

Determination of Olanzapine in Five Different Pharmaceutical Formulations by LC-MS Method

Mevlut A[1]*, Yucel K[2], Mehmet Emrah Y[2] and Onur S[2]

[1]Department of Medical Laboratory Techniques, Health Services Vocational Training School, Ataturk University, 25240, Erzurum, Turkey
[2]Department of Analytical Chemistry, Faculty of Pharmacy, Ataturk University, 25240, Erzurum, Turkey

Abstract

A new, simple, inexpensive, accuracy, sensitive and rapid liquid chromatography/mass spectrometry (LC/MS) method for the determination of olanzapine in pharmaceutical preparations was developed and validated. In order to carry out this study, method parameters were optimized to be 1 mL/min flow rate, 25°C column temperature, injection volume of 10 µL. Irbesartan was selected as internal standard. Chromatographic separation was carried out on reverse phase, Agilent C18 column (5 µm, 150 × 4,6 mm). It was used water containing 0.1% TFA: acetonitrile (90:10, V/V) in isocratic elution as mobile phase. Quantitative analyses were operated using the selected product ions for olanzapine (m/z 313.3) and irbesartan (m/z 429.2) in positive ion mode, 100 V fragmentor voltage. The proposed method was validated in terms of linearity, specificity, LOD, LOQ, accuracy, precision and recovery. Method was linear between 2-300 ng/mL. %CV and bias of the method was lower than 7.55% and 7.59%, respectively. Correlation coefficient (r) was found to be 0.999. LOQ and LOD values was 2 ng/mL and 0.7 ng/mL, respectively. The intra-day and inter-day precisions were less than 7.55%, and the intra-day and inter-day accuracies were found between 4.95 and 7.59%. The method was successfully applied into five different pharmaceutical formulations for the determination of olanzapine. Analytical recovery was 102.4% and pharmaceutical formulations were successfully quantitated.

Keywords: LC-MS; Olanzapine; Validation; Pharmaceutical formulations

Introduction

Olanzapine (Figure 1) which is named as 2-methyl-4-4-(4-methyl-1-piperazynyl)-10H-thio (2,3-b) (1,5) benzodiazepine is used as a pharmaceutical formulation since 1985. Olanzapine is an atypical antipsychotic drug that has a great affinity to dopamine (D1, D2, D3, D4), serotonin (5HT-2A, 5HT-2C, 5HT3, 5HT6), muscarinic (M1, M5) histamine H1 and adrenergic receptors. Olanzapine has been demonstrated for alleviating positive and negative symptoms of schizophrenia with a relatively low occurrence of extrapyramidal side effects. By this way it can differentiated from the unselective typical antipsychotics which block the both striatal and limbic neurons and cause adverse effects [1,2]. Olanzapine is approved in the US and Europe for the oral treatment of schizophrenia and bipolar I disorder within the dose range of 5-20 mg/day [3]. There are recently many pharmaceutical formulations of olanzapine, which have been widely used in psychiatry. Thus, new, simple, rapid, sensitive, less time consuming and cost-effective analytical methods for the estimation of olanzapine in pharmaceutical formulations are necessary for the routine analysis. According to our survey of literature, this study is the first study to determine olanzapine from five different pharmaceutical formulations. Several studies using various analytical methods for analysis of olanzapine in the pharmaceutical preparations are described in the literature. These methods include the instrumental techniques such as LC/MS [4,5], LC-MS/MS [6-9] high performance liquid chromatography (HPLC) [10-16], spectrophotometry [10,17-23] and voltammetry [17,24]. In 1960s and 1970s, new methods were invented for HPLC and these findings were applied on pharmaceuticals [25]. Liquid chromatography became a crucial part in quality control studies of drugs. It is one of the most accepted method for drug analysis [26]. In this study, it is aimed to develop and validate an accurate, new, simple, precise, sensitive LC-MS method to determine olanzapine in five different pharmaceutical formulations. The proposed method was validated with respect to the FDA guideline [27].

Materials and Methods

Chemicals

Olanzapine and internal standard (irbesartan) purchased from Sigma-Aldrich (St, Louis, Mo, USA). HPLC grade methanol and trifluoroacetic acid (TFA) were obtained from Merck Germany. The mobile phase and solution were prepared in deionized water, which was prepared daily, filtered (0.45 µm) and degassed by sonicator in laboratory. Tablet formulations of olanzapine located in the Turkish pharmaceutical market (Ollafax, Rexapin, Olaxinn, Ozaprin, Zyprexia) were obtained from pharmacy. Olanzapine stock solutions were also prepared daily in methanol.

Apparatus

Chromatographic determinations performed by an Agilent Technologies 1200 Series HPLC system conducted with a Mass detector (Agilent G1314B MSD), a degasser system (Agilent G1322A), a quat pump (Agilent G1311A), an autosampler (Agilent G1329A ALS) and a reserved phase C_{18} HPLC column (5 µm, 250 × 4.6 mm) (Agilent, USA).

Chromatographic and mass spectrometric conditions

The mobile phase was a mixture of TFA 0.1% in deionized water

***Corresponding author:** Mevlut Albayrak, Department of Medical Laboratory Techniques, Health Services Vocational Training School, Ataturk University, 25240, Erzurum, Turkey, Tel: +904422316073; E-mail: m_albayrak25@hotmail.com

Received September 05, 2018; **Accepted** September 17, 2018; **Published** September 21, 2018

Citation: Mevlut A, Yucel K, Emrah YM, Onur S (2018) Determination of Olanzapine in Five Different Pharmaceutical Formulations by LC-MS Method. J Chromatogr Sep Tech 9: 409. doi: 10.4172/2157-7064.1000409

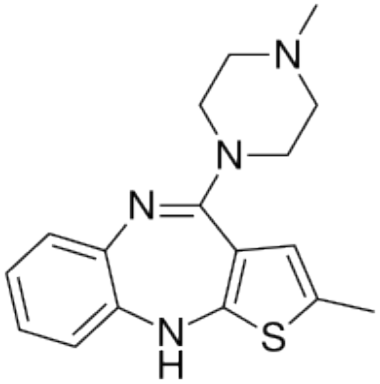

Figure 1: Chemical structure of Olanzapine.

(A) and acetonitrile (B). An isocratic mobile phase consisting of A-B (20:80, v/v) was used during the analysis. The flow rate of the mobile phase was 1 mL/min, the column temperature was variable according to room temperature and the amount of injected sample was 10 µL. The mass spectrometer was operated in positive ion electrospray mode. The capillary sprayer voltage was 2.0 kV and the fragmentation voltage were 100 V for both olanzapine and irbesartan. The source temperature was 250°C and the drying gas temperature was 300°C. The drying gas flow rate was set to 10 mL/min^{-1}. The investigated mass range is m/z 100-700 at 0.5 sec per scan with a 0.1 sec inter scan delay in scan monitoring mode (SCAN). Quantitative analysis, carried out in Selective-Ion Monitoring (SIM) mode, detected olanzapine at m/z 313.3 and the Internal Standard (IS), irbesartan, at m/z 429.2, all in the form of ions. The quantitation calculations were performed accordance with peak area ratio of olanzapine to IS for different concentrations.

Preparation standard and sample solutions

Stock solutions of olanzapine and irbesartan were prepared by dissolving accurately weighed amounts of each reference compound in methanol to yield concentrations of 200 µg/mL for olanzapine, and 1 mg/mL for irbesartan. These stock solutions were stored at +4°C. Working solutions for LC/MS method were prepared by diluted from standard reference olanzapine solution in methanol containing to 50 ng/mL of irbesartan as internal Standard. Five different pharmaceutical preparations specified to contain olanzapine were used as real sample. Ten tablets were weighted accurately and average weight per tablet was calculated. Tablets were ground to a fine powder and solutions on three different concentrations were prepared for each sample including 50 ng/mL IS. The confidence intervals of the method were determined by recovery study.

Validation parameters

The process confirm that the analytical procedure employed for the analysis is suitable for its intended use and to show reliability of the method. In this study, the validation parameters which are accuracy, precision, linearity, limit of detection (LOD), limit of quantification (LOQ) recovery for quantitative analysis of olanzapine in tablets were tested and validation data were evaluated according to validation acceptance criteria (FDA).

Results and Discussion

Method development and optimization

In order to obtain the most optimum conditions for chromatograms, different chromatographic parameters were tested. The optimized LC/MS conditions were provided in Section 4.3. Under the described chromatographic conditions, retention times were 1.51 min for olanzapine and 2.15 min for IS. In the mass spectrometric analysis carried out via intensity of product ion. The molecular ions had more high intensity than the others and those ions were selected product ions for olanzapine (m/z 313.3) and irbesartan (m/z 429.2) (Figure 2). Those spectrums were obtained by selection of ions with mass 429.2 until the 2 minute and with mass 313.3 after 2 minutes in the SIM mode.

Validation: Linearity, precision, accuracy and recovery

Calibration curves were constructed for olanzapine standard by plotting the concentration of olanzapine versus peak area response ratio of the internal standard peak area. Calibration curves of olanzapine appeared linear in the concentration range of 2 to 300 ng/mL (2, 10, 25, 75, 100, 150, 250 and 300 ng/mL). The correlation coefficient (r) of all the calibration curves were consistently greater than 0.999 (Figure 3). Precision and accuracy of our LC-MS method are shown in Table 1. The quality control (QC) samples were prepared by adding aliquots of standard working solution of olanzapine to final concentrations of 5, 50 and 200 ng/mL with 50 ng/mL IS of each. The accuracy of the method was stated as Relative Error% (RE). For all the concentrations of olanzapine studied, intra-day and inter-day Relative Standard Deviation (RSD) values were ≤ 7.55%, and for all concentrations of olanzapine the relative errors were between 4.95 and 7.59%. Six extraction replicates were measured to calculate %RSD and bias.

Limits of detection (LOD) and quantitation (LOQ)

The LOD and LOQ of olanzapine for LC/MS measurements were determined by injecting a dwindling concentrations of the olanzapine standard solution under the linear range of the calibration curve. The lowest concentrations determined where the signal/noise ratio was at least 10:1, this concentration was regarded as LOQ value. The LOD value was described as a signal/noise ratio of 3:1. The LOD and LOQ values for the method were 0.7 and 2 ng/mL, respectively.

Recovery test by standard addition method

Developed and validated method was performed on five different pharmaceutical formulation of olanzapine. Drug solutions were prepared at 25 ng/mL concentration as described in section 2.4 and they were analyzed with 50 ng/mL IS. There were no interfering peaks affecting quantification of olanzapine and IS. Specificity were evaluated by placebo tablet formulation. Analytical recovery analysis was performed by adding known concentrations of olanzapine standard solution (5, 50 and 200 ng/mL) to the 25 ng/mL concentrations of pre-analyzed samples of commercial dosage forms. Analytical %recovery values assessed by comparing the actual concentration of sample and analyse results in accordance with the method. The results were given as percentage in Table 2.

Application of proposed method

The proposed method was applied successfully for the determination of olanzapine in five different pharmaceutical formulations (Ollafax, Rexapin, Olaxinn, Ozaprin, Zyprexia). The results in Table 3 and Figure 4 showed that the proposed method is suitable for the determination of olanzapine pharmaceutical formulations and the excipients in the dosage forms do not interfere. A new LC-MS method was developed and validated for the quantitative determination of olanzapine in pharmaceutical preparations. The estimated calibration range was 2-300 ng/mL with practically no interference or matrix effects from pharmaceutical preparation media. Validation showed our method was

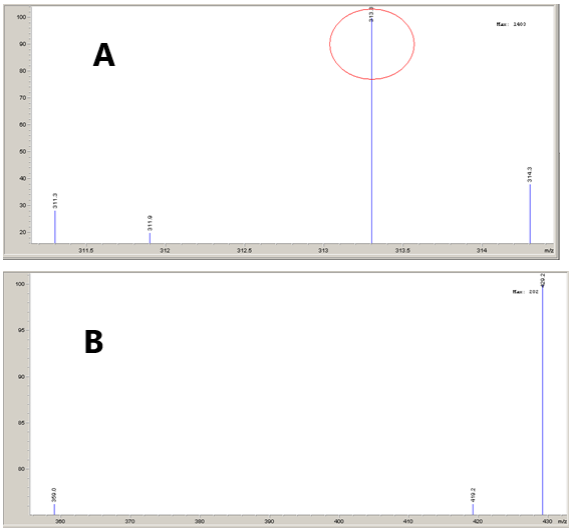

Figure 2: Mass spectrum of selected ions A) Olanzapine B) Irbesartan.

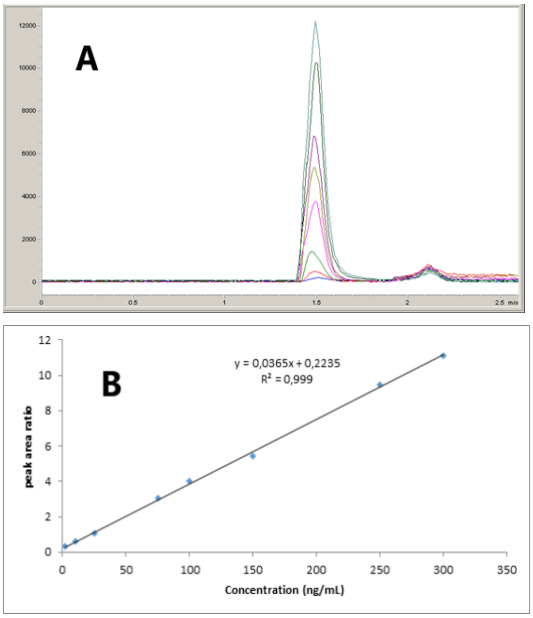

Figure 3: Measurements for olanzapine standard solutions in linear range A) Overlapped LC/MS chromatograms for linear range B) Calibration curve.

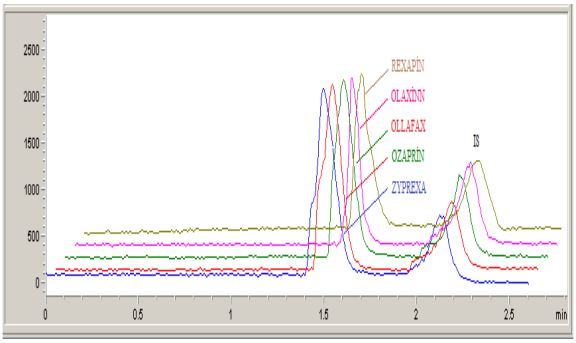

Figure 4: LC/MS chromatograms of five different tablet solutions prepared at 25 ng/mL and 50 ng/mL of IS.

successful in measuring olanzapine with high accuracy and acceptable RSD values. The mean recovery level was found as 102.4%. Compared to previously reported methods, proposed method provided higher throughput and much better sensitivity despite measurements at very low concentrations with its simple sample preparation and short run time. Some of previous studies for determination of olanzapine has derivatization procedure. There is no derivatization in proposed method and we didn't use any buffer solution to control pH value of solutions. Moreover, as an advantage of the mass spectrometer, developed method can be performed with more selective for olanzapine. According to our literature survey, this study is the first study to determine olanzapine from five different commercial forms.

Conclusion

The obtained results demonstrated that a new, accurate, sensitive and rapid LC-MS method was successfully developed and validated for the determination of olanzapine in bulk and pharmaceutical formulations. This proposed method could be trustfully used in routine analysis of olanzapine in quality control laboratories.

Acknowledgements

The authors are grateful to the University of Ataturk for the financial support of this work (Project No: 2013/273). This work was presented as a poster on 9th Aegean Analytical Chemistry Days.

Added (ng/mL)	Intra-day			Inter-day		
	Mean ± SD (ng/mL)	bias	% RSD	Mean ± SD (ng/mL)	bias	% RSD
5	5.32 ± 0.33	6.37	6.15	5.38 ± 0.40	7.59	7.48
50	53.75 ± 2.90	7.50	5.40	52.71 ± 3.98	5.43	7.55
200	210.19 ± 5.56	5.10	2.65	209.89 ± 5.32	4.95	2.54

SD: Standard Deviation, RSD: Relative Standard Deviation (n=6).

Table 1: Intra-day and Inter-day precision and bias values.

Parmaceutical Preparation	Added (ng/mL)	Mean ± SD (µg/mL)	Recovery %	RSD %
Olaxinn 25 ng/mL	5	32.96 ± 0.76	109.87	1.02
	50	74.88 ± 3.31	99.84	0.54
	200	225.62 ± 1.62	100.28	1.71
Ollafax 25 ng/mL	5	29.41 ± 2.20	98.06	1.07
	50	74.59 ± 0.53	99.46	2.01
	200	228.23 ± 5.39	101.43	1.04
Ozaprin 25 ng/mL	5	32.80 ± 0.59	109.34	3.02
	50	75.22 ± 2.43	100.29	1.23
	200	229.64 ± 12.47	102.06	0.05
Rexapin 25ng/mL	5	31.91 ± 2.32	106.34	2.01
	50	71.37 ± 1.96	95.03	0.03
	200	222.41 ± 1.24	98.85	1.01
Zyprexa 25 ng/mL	5	32.29 ± 2.10	107.64	1.06
	50	79.25 ± 2.01	105.67	0.42
	200	228.83 ± 11.75	101.70	2.05

Table 2: Recovery test of olanzapine (by standard addition method).

Commercial Preparation (10 mg)	Found SD (mg)	Recovery %	RSD %
Rexapin	9.61 ± 0.32	96.13	3.32
Olaxinn	9.53 ± 0.05	95.34	0.53
Ozaprin	9.39 ± 0.23	93.92	2.45
Ollafax	9.34 ± 0.34	93.40	3.64
Zyprexa	9.78 ± 0.14	97.82	1.43

Table 3: Determination of olanzapine in five different pharmaceutical formulations.

Conflict of Interest

The authors declare that they have no conflicts of interest to disclose.

References

1. Callaghan JT, Richard FB, Louis RP, Charles MB (1999) Olanzapine. Clinical Pharmacokinetics 37: 177-193.
2. Ceylan ME (2001) Araştırma ve klinik uygulamada biyolojik psikiyatri şizofreni. AstraZeneca.
3. Bhana N, Rachel HF, Roger O, Greg LP (2001) Olanzapine. Drugs 61: 111-161.
4. Tumpa A, Stajic A, Jancic-Stojanovic B, Medenica M (2017) Quality by Design in the development of hydrophilic interaction liquid chromatography method with gradient elution for the analysis of olanzapine. J Pharm Biomed Anal 134: 18-26.
5. Zhuang T, Zhang W, Cao L, He K, Wang Y, et al. (2018) Isolation, identification and characterization of two novel process-related impurities in olanzapine. J Pharm Biomed Anal 152: 188-196.
6. Michely JA, Maurer HH (2018) A multi-analyte approach to help in assessing the severity of acute poisonings-Development and validation of a fast LC-MS/MS quantification approach for 45 drugs and their relevant metabolites with one-point calibration. Drug Test Anal 10: 164-176.
7. Miroshnichenko II, Baymeeva NV (2018) Simultaneous Determination of Antipsychotic Drugs and Their Active Metabolites by LC-MS-MS and its Application to Therapeutic Drug Monitoring. J Chromatogr Sci 56: 510-517.
8. Cánovas M, Torres F, Domenech G, Cebrecos J, Pelagio P, et al. (2011) Bioequivalence evaluation of two dosage forms of olanzapine 10 mg formulations in healthy volunteers. Arzneimittelforschung 61: 75-79.
9. Singhal R, Thakkar V, Srivastava A (2011) Evaluation of bioequivalence of two oral formulations of olanzapine. Indian Journal of Pharmaceutical Sciences 73: 678-682.
10. Bıryol I, Erk N (2003) Voltammetric, spectrophotometric and high-performance liquid chromatographic analysis of olanzapine. Analytical Letters 36: 2497-2513.
11. Rao RN, Raju AN, Narsimha R, Babu GR (2008) Isolation and characterization of process related impurities of olanzapine using HPLC and ESI-MS/MS. Journal of Separation Science 31: 107-118.
12. Shah CR, Shah NJ, Suhagia BN, Patel NM (2007) Simultaneous assay of olanzapine and fluoxetine in tablets by column high-performance liquid chromatography and high-performance thin-layer chromatography. Journal of AOAC International 90: 1573-1578.
13. Prameela R, Bala S (2009) Development of HPLC method for the determination of olanzapine in bulk and dosage forms. Int J Pharm Tech Res 1: 654-657.
14. Reddy BV, Suresh Reddy KVN, Sreeramulu J, Kanumula GV (2007) Simultaneous determination of olanzapine and fluoxetine by HPLC. Chromatographia 66: 111-114.
15. Pathak A, Rajput S (2009) Development of a stability-indicating HPLC method for simultaneous determination of olanzapine and fluoxetine in combined dosage forms. J Chromatogr Sci 47: 605-611.
16. Basavaiah K, Anil Kumar UR, Tharpa K (2008) Quantitative determination of olanzapine in pharmaceutical preparations by HPLC. Journal of the Mexican Chemical Society 52: 120-124.
17. Raggi MA, Casamenti G, Mandrioli R, Izzo G, Kenndler E (2000) Quantitation of olanzapine in tablets by HPLC, CZE, derivative spectrometry and linear voltammetry. J Pharm Biomed Anal 23: 973-981.
18. Tantawy MA, Hassan NY, Elragehy NA, Abdelkawy M (2013) Simultaneous determination of olanzapine and fluoxetine hydrochloride in capsules by spectrophotometry, TLC-spectrodensitometry and HPLC. J Adv Res 4: 173-180.
19. Kumar RS, Gayathri P, Duganath N, Kiran C, Sridhar C (2011) Simultaneous Estimation of Fluoxetine HCl and Olanzapine in Bulk Drug and Pharmaceutical Formulation by Using UV-Visible Spectroscopy Method. Int J Pharma Sci Drug Res 3: 52-55.
20. Rego do JF, Moura de JI, Moita GC (2010) Spectrophotometric olanzapine determination in pharmaceutical formulations: method development and validation. New Chemistry 33: 471-477.
21. Rajendraprasad N, Basavaiah K (2010) Highly sensitive spectrophotometric determination of olanzapine using cerium (IV) and iron (II) complexes of 1, 10-phenanthroline and 2, 2′-bipyridyl. Journal of Analytical Chemistry 65: 482-488.
22. Rajendraprasad N, Basavaiah K (2009) Determination of olanzapine by spectrophotometry using permanganate. Brazilian Journal of Pharmaceutical Sciences 45: 539-550.
23. Patel VM, Patel JA, Havele SS, Dhaneshwar SR (2010) First and Second Derivative Spectrophotometric Methods for Determination of Olanzapine in Pharmaceutical Formulation. International Journal of ChemTech Research 2: 756-761.
24. Yilmaz B, Albayrak M (2014) Determination of Olanzapine in Pharmaceutical Preparations by Square Wave and Differential Pulse Voltammetric Methods. Lat Am J Pharm 33: 595-600.
25. Misiuk W (2010) The role of assay methods in characterizing the quality of bulk pharmaceuticals. J Pharm and Bioallied Sci 2: 88-92.
26. Hofer JD, Olsen BA, Rickard EC (2007) Is HPLC assay for drug substance a useful quality control attribute? J Pharm Biomed Anal 44: 906-913.
27. USFDA (2001) Guidance for industry: Bioanalytical method validation. Food and Drug Administration.