Int J Ayu Pharm Chem

RESEARCH ARTICLE

www.ijapc.com

e-ISSN 2350-0204

Qualitative and Quantitative Estimation of Agomelatine from Rat Brain Tissue by a Hyphenated Chromatographic Technique (LC-MS)

Jignesh S Patel[1*], Girish K Jani[2], Akshay H Shah[3], Pinak R Patel[4] and Mayank Bapna[5]

[1]School of Pharmacy, R K University Rajkot, Kasturbadham, Rajkot, Gujarat, India
[2]Department of Pharmaceutics, SSR College of Pharmacy, Silvassa, Dadar and Nagar Haveli, India
[3] Department of Pharmacology, Indubhai Patel College of Pharmacy and Research Centre, Dharmaj, Gujarat, India
[4] Department of Pharmaceutical Analysis, Smt. Shah Pharmacy College, Mahemdabad, Gujarat, India
[5]Department of Pharmaceutical Chemistry, Indubhai Patel College of Pharmacy and Research Centre, Dharmaj, Gujarat, India

Abstract

A hyphenated analytical technique was developed and validated for qualitative and quantitative determination of Agomelatine from rat brain tissue. Chromatographic conditions utilized Zorbax XDB-CN (100*2.1 mm) as separation medium and combination of methyl alcohol with 0.2% formic acid in water as the elution medium. Detection was achieved with positive multiple reaction monitoring transitions m/z 244.41/185.41 and m/z 315.962→270.00 for Agomelatine and Clonazepam (IS), respectively. The lower limit of quantification was 1 ngmL^{-1}and the linear calibration range was from 1 to 100ngmL−1. The mean recovery for Agomelatine and IS was 81.02 and 82.52% in brain respectively. The method has been successfully applied to the pharmacokinetic studies of agomelatine in brain tissue after intranasal administration

Keywords *Hyphenated technique, Agomelatine, Rat Brain, Nose to brain delivery system*

Greentree Group

Received 30/03/16 Accepted 20/06/16 Published 10/07/16

INTRODUCTION

The active ingredient Agomelatine is pharmacologically an active anti-depressive agent having high agonistic action on melatonin receptors and antagonistic action on 5-HT receptors. Chemically is acetamide derivative with naphthyl moiety more than 80% of drug absorbed after oral administration[1]. But the major drawback is minimal absolute bioavailability of drug, about 5%, which also has huge variation amongst individuals due to relative difference in Cytochrome P4501A2 activity and first pass metabolism[2].

As this class has anti-depressant action, the effect of drug must be observed within shorter time but the highest concentration is achieved after 1-2 hours of oral administration. So, for quick onset of action the drug must reach to the CNS directly for maximum antidepressant activity and must avoid 1st pass metabolism. Such can be assured by nose to brain delivery[3]. There are so many hyphenated tandem chromatographic methods reported for qualitative analysis of melatonin from human plasma[4-5], dog plasma[6-7] and milk[8]. As per in-depth literature review, no method is evident for qualitative and quantitative analysis of Agomelatine from brain tissue after nose to brain delivery. Therefore the primary objective of the research was to develop an extraction method for recovering Agomelatine from rat brain tissue followed by quantification of brain uptake by a rapid LC-MS method.

EXPERIMENTAL WORK

Chemicals and reagents

Agomelatine was obtained as a gift sample from Enaltec Labs, Igatpuri, Nashik, India. having purity of 99.76%. High purity mili-Q grade water was purchased from Millipore, Bangalore, India). LC grade methyl alcohol and formic acid were purchased from Merck (Mumbai, India).

Experimental Conditions

Initial method was developed by utilizing pure standard solution of Agomelatine (100 ng/ml) on Agilent 1290 infinity instrument having Zorbax XDB-CN (100*2.1 mm) as separation medium and combination of methyl alcohol with 0.2% formic acid in water as the elution medium at a flow rate of 0.5 ml/min for total 4 minutes. Mass detection of elute was carried out on triple quad 4500 mass spectrometer in positive ionization mode using ESI as the ionization source with spray voltage of 5500 V and 550°C as the source temperature. Collision energy was 38eV and delustering potential

was 58V. Detection was achieved with positive multiple reaction monitoring transitions m/z 244.41/185.41 and m/z 315.962→270.00 for Agomelatine and Clonazepam (IS), respectively

Preparation of Standards Samples

Stock solutions of 1mg/mL was prepared by dissolving appropriate amount Agomelatine in methanol. Spiking solutions of different concentrations were prepared using respective stocks, for calibration curve using methanol with 0.2% formic acid in water use as a diluent. Six calibration curve standards from 1 ng/mL to 100ng/mL were prepared for Agomelatine in rat human plasma and brain tissue homogenate. All stock solutions were stored at 2-8°C and spiked samples were stored at -70°C.

Preparation of calibration curve

At the time of analysis, the samples were removed from the deep freezer and kept at room temperature and allowed to thaw. A 100 µL aliquot of Clonazepam (I.S) solution was added to 500 µL of plasma containing the drug sample. To final sample 500 µl acetonitrile was added to precipitate the plasma protein and to ensure proper mixing. The protein precipitating step was followed by the addition of 1 ml ethyl acetate and this mixture was again vortexed for proper extraction of the drug. The mixture was then centrifuged at 5000 rpm for 10 minutes The supernatant was taken out and evaporated under a nitrogen stream. The left residue was dissolved in the 250µl mobile phase and a 20µL aliquot of the reconstituted extract was injected for analysis.

Recovery

The recovery of AGM from spiked human plasma and brain homogenate samples subjected to protein precipitation was determined at three concentration levels – 1, 50 and 100 ng/mL for AGM repeated analysis (n = 3). The recovery of the analytes was calculated by comparing the analyte/IS peak area ratio of processed plasma samples with the corresponding ratio obtained from the processed aqueous solutions at the same concentrations. Recovery of the IS was also evaluated by calculating the peak area ratio of the IS from the spiked processed plasma samples and aqueous solutions at equivalent concentrations.

Stability

Stability in human plasma and brain homogenate of AGM at low, medium and high concentration levels, at room temperature for 3 hours, -70°C for 2 weeks in order to simulate sample handling and storage time in the freezer before analysis. The stability of the drug-spiked human

plasma and brain homogenate samples at three levels was investigated for three freeze–thaw cycles. The stability was assessed by comparing the mean concentrations of the stability sample with theoretical concentrations.

Brain Pharmacokinetic Studies

Animal dosing and sample collection for the brain pharmacokinetic study,

Wistar rats (200-270 g) were divided into two groups. Group I was administered with Agomelatine solution intravenously and Group II received the Agomelatine loaded microemulsion . For intranasal dosing, the supine position of rats was insured and a volume of 20 µl was instilled in nostril with the help of a micropipette (5-50 µl capacity). For intravenous administration of 20 µl Agomelatine solution, rat tail vein was exploited. 1 ml of blood samples were collected into the heparinized tubes at a time interval of 0, 0.5, 1, 2, and 4 hours and for each time interval, 3 rats were allocated. After collecting the blood samples, respective animals were sacrificed to collect brains.

Extraction of Agomelatine from plasma

The plasma was separated by centrifuging, the collected blood at 5000 rpm for 15 minutes. 100µl of 1µg/ml of Clonazepam (internal standard) was added in 500 µl of plasma. Then, 500 µl acetonitrile was added to precipitate the plasma protein and to ensure proper mixing, Remi CM-101 cyclomixer (Mumbai) was used. The protein precipitating step was followed by the addition of 1 ml ethyl acetate and this mixture was again vortexed for proper extraction of the drug. The mixture was then centrifuged at 5000 rpm for 10 minutes The supernatant was taken out and evaporated under a nitrogen stream. The left residue was dissolved in the mobile phase and a 20 µL aliquot of the reconstituted extract was injected for analysis[9].

Extraction of Agomelatine from brain homogenate

Rat brain was isolated by following regulatory standards of CPCSEA guidelines. The integrity of brain was ensured during its removal and weighed before preparing the homogenate. The weighed brain was homogenized with normal saline in a test tube placed on a cold ice bath. The total brain homogenate obtained so far was transferred into a 15ml falcon tube. Then, to this homogenate, 100µl of internal standard (internal standard) and 1ml of 20% trichloroacetic acid (deproteinizing agent) were added. This mixture was centrifuged at 5000 rpm for 15 minutes and the supernatant was treated similarly to the above-

mentioned extraction procedure of drug from the plasma[10].

Pharmacokinetic analysis

The plasma and brain concentrations of Agomelatine at different time interval were calculated from the LC-MS data and various pharmacokinetic parameters were determined by a pharmacokinetic software (PK solver Microsoft Excel)

The area under the curve, determined by software was used to calculate direct transport percentage (DTP) and drug targeting efficiency % (DTE%) of Agomelatine into the brain.[10]

$$DTP = [\{(AUC_{brain})^{i.n} - (AUC_{\times})\}/(AUC_{brain})^{i.n}] \times 100$$

$$\text{Where , } \quad AUC_X = \frac{(AUC_{brain})^{i.v}}{(AUC_{plasma})^{i.v}} \times (AUC_{plasma})^{i.n}$$

AUC_X is AUC fraction of brain contributed by systemic circulation across the blood brain barrier following intranasal drug administration.

$$DTE\% = [(\frac{AUC_{brain}}{AUC_{blood}})^{i.n}/(\frac{AUC_{brain}}{AUC_{blood}})^{i.v}] \times 100$$

Table 1 Statistical data of Agomelatine for linearity study

In plasma		In brain tissue	
Sample size	6	Sample size	6
Concentration	1 -100 ng/ml	Concentration	1-100 ng/ml
Intercept	0.041	Intercept	0.0473
Slope	0.0437	Slope	0.045
Regression equation	Y=0.0437x +0.041	Regression equation	Y=0.045x +0.0473
Highest standard concentration (ng/ml)	100 ng/ml	Highest standard concentration (ng/ml)	100 ng/ml

RESULTS AND DISCUSSION

When method was operated under optimized conditions, the retention time for Agomelatine and Clonazepam was found to be 2.27 min and 2.82 min and peak was monitored at 244.41/185.41 Da and 315.962→270.000, respectively in-mass spectrometer.

Linearity

The linearity of method was studied in the range of 1-100ng/ml as, below 1 ng/ml concentration significant reduction in S/N ratio is observed. The highest upper range with which work was carried out was 100ng/ml. The observed regression coefficient was found to be in acceptance criteria for the selected range (Figure 1 & 2). All the statistical parameters are highlighted in table 1. When blank sample of plasma and brain tissue is analysed (without spiked drug), the chromatogram did not show any interference at the base line level. Hence matrix effect is not evident (Figure 3 and 4).

Recovery

The extraction recoveries of Agomelatine were 80.2, 79.77 and 83.1% for QC samples at concentrations of 1, 50 and 100 ng mL^{-1},

respectively in brain tissue and 81.04, 80.6 and 85.3 for QC sample at 1, 50 and 100ng mL^{-1}, respectively in plasma samples. The mean recovery for Agomelatine was 81.03 in brain tissue and 82.31 in plasma.

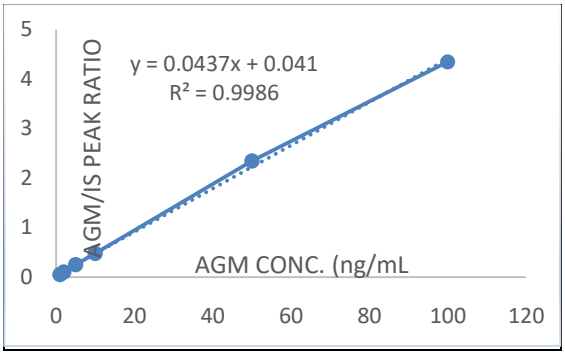

Figure 1 Standard curve of AGM in plasma

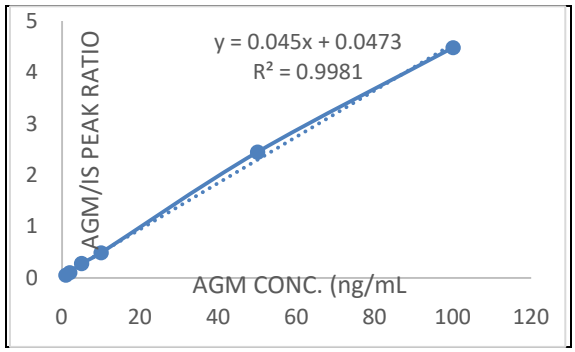

Figure 2 Standard curve of AGM in brain tissue

The extraction recovery of IS was also evaluated and the mean recovery was 82.52%. and 84.4% in brain tissue and plasma respectively. Extraction recoveries for AGM and IS in Rat plasma and brain tissue is shown in table 2**.**

Table 2 Stability of agomelatine in plasma sample and brain tissue

Plasma sample			Brain homogenate		
Spiked Conc. (ng mL^{-1})	**Conc.found (ng mL^{-1})**	**% Accuracy**	**Spiked Conc. (ng mL^{-1})**	**Conc. found (ng mL^{-1})**	**% Accuracy**
Short term stability (about 25 ^{0}C, 8 h)					
1	0.97± 0.002	97.0	1	0.984± 0.02	98.4
50	48.8± 0.03	97.6	50	48.2± 0.3	96.4
100	98.45±2.78	98.45	100	99.12±2.78	99.12
Long term stability (-70 ^{0}C, 14 days) and freeze–thaw stability					
1	0.98±.001	98	1	0.992±.001	99.2
50	48.4±.04	96.8	50	48.7±.04	97.4
100	98.34±3.46	98.34	100	99.5±3.46	99.5

Stability

In the study, Agomelatine was stable was stable after being placed at room temperature (about 25^{o}C) for 3hrs and after undergoing three freeze–thaw cycles during frozen storage at -80 ^{0}C for 14 days. Stability data are summarized in Table 3. This stability study showed that Agomelatine had good stability in plasma and brain tissue.

Table 3 Extraction recoveries for AGM and IS in Rat plasma and brain tissue

Analyte	Plasma	Brain tissue
AGM	82.31 ± 1.47	81.02 ±1.478
IS	84.4 ±2.32	82.52 ± 2.57

Brain pharmacokinetic studies

The accuracy, recovery, matrix effect and stability tests all met the requirements for the quantitative determination in biological samples. This method was applied for quantification of Agomelatine from brain tissue after dosing through IN and IV route.

The pharmacokinetic parameters (Cmax, Tmax, AUC0-8h, AUC0-∞, Kelim and half-life) of Agomelatine were assessed after single dose of I.V and I.N administration and results are provided in Table 4**.**

After I.N administration of Agomelatine microemulsion the AUC0-8h in brain and plasma were found to be 1028.33 and 521.42 ng.h/ml respectively. In this case at each time point, the concentration of Agomelatine was comparatively higher in brain than plasma. In this study, intranasal route for Agomelatine was compared with intravenous route and the results show 1.97 folds enhanced bioavailability of Agomelatine in brain when agomeltine was administered intranasally.

Table 4 Pharmacokinetic profile of different formulation after dosing the Wistar rats intranasally and intravenously

Formulation	Sample	Cmax (ng/mL)	Tmax (h)	AUC_{0-T} (ng.h/mL)	$AUC_{0-\infty}$ (ng.h/mL)	Kelim (h^{-1})	$t_{1/2}$ (h)	DTE %	DTP %
AGM microemulsion	Brain	227.79	2	1028.33	1185.82	0.276	2.51		
AGM solution	Brain	107.72	0.5	405.55	521.42	0.202	3.42	84	505.3

The drug targeting efficiency and direct transport percentage of Agomelatine microemulsion were calculated as 505.3% and 84% respectively. These results was similar to established fact of selective brain targeting via intranasal route.

CONCLUSION

The developed LC-MS method was found to be quick, specific and reliable for qualitative and quantitative evaluation of Agomelatine from rat brain tissue after nose to brain delivery of nano-emulsion. The method was also found to be highly specific as there is no interference imposed by matrix in determination of Agomelatine. Furthermore, this method can be also applied for determining pharmacokinetic behavior of the Agomelatine. this method was successfully applied for determination of Agomelatine reaching to the brain tissue after nose to brain delivery of microemulsion containing same active substance.

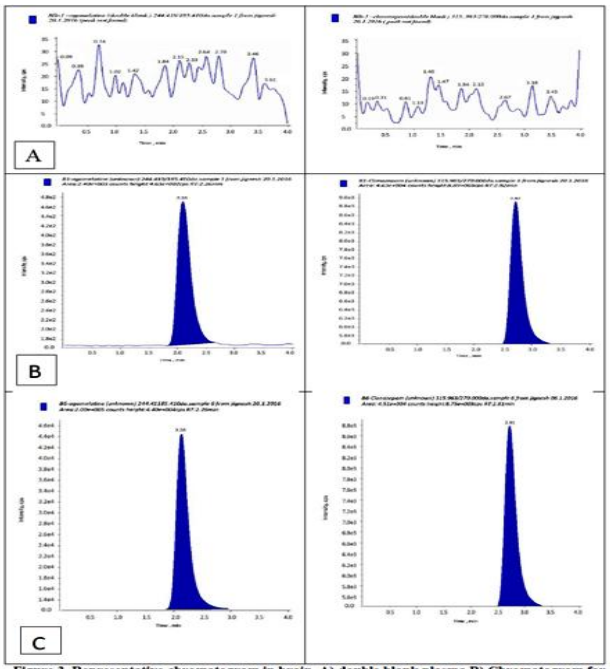

Figure 3. Representative chromatogram in brain. A) double blank plasma B) Chromatogram for AGM at 1 ng/mL and IS at 50 ng /mL. C) Chromatogram for AGM at 100 ng/mL and IS at 50 ng /mL

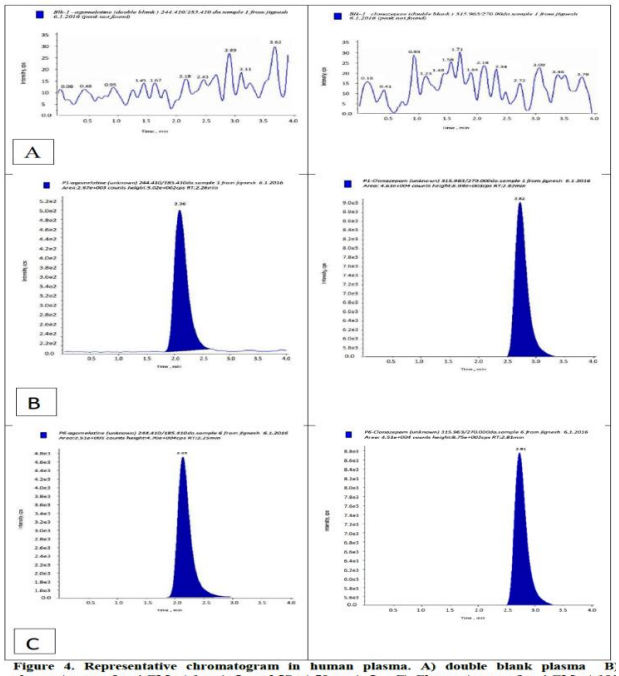

Figure 4. Representative chromatogram in human plasma. A) double blank plasma B chromatogram for AGM at 1 ng/mL and IS at 50 ng /mL. C) Chromatogram for AGM at 10 ng/mL and IS at 50 ng /mL

ACKNOWELGMENT

Authors are extremely grateful to Enaltec Labs, Igatpuri, Nashik, India for providing Agomelatine as a gift sample. The authors are also grateful to department of bioscience, V.V nagar for providing excellent facilities for carrying out this research work. Authors also extend their sincere thanks to RK University for promoting research studies.

REFERENCES

1. Zupancic M, Guilleminault C (2006). Agomelatine: a preliminary review of a new antidepressant CNS Drugs. Pharmaceutical Science and Bio Scientific Research, 20(12) : 981-92 .

2. Manikandan S(2011). Agomelatine: A novel melatonergic antidepressant. J Pharmacol Pharmacother, 2(1): 122-123.

3. McAllister-Williams RH, Baldwin DS, Haddad PM and Bazire S (2012). The use of antidepressants in clinical practice: focus on Agomelatine. Human Psychopharmacology, 25(2): 95–102, .

4. Patil SR, Nerurkar KK, Kalamkar AM, Pukale V, Mangaokar KV, Pingale SG(2012). Validated LC–MS/MS method for quantification of Agomelatine in human plasma and its application in a pharmacokinetic study. Journal of Mass spectrometry, 47(3) : 23-48.

5. Carter MD, Calcutt MW, Malow BA, Rose KL, Hachey DL(2012). Quantitation of melatonin and n-acetylserotonin in human plasma by nanoflow LC-MS/MS and electrospray LC-MS/MS. J Mass Spectrom, 47(3): 277-285.

6. Chopade V V, Tembhurkar N B, Jadhav S B et al (2012). Development and Validation of A Stability Indicating Assay Method of Mefloquine hydrochloride by Using Different Stress Degradation Conditions. Journal of Pharmacy Research, 5(5):111-115.

7. McAllister-Williams RH, Baldwin DS, Haddad PM and Bazire S (2010). The use of antidepressants in clinical practice: focus on Agomelatine. Human Psychopharmacology, 25(3) : 95–102 .

8. Karunanithi D, Radhakrishna A, Sivaraman KP, Biju VM(2014). Quantitative determination of melatonin in milk by LC-MS/MS. J Food Sci Technol, 51(4): 805-812.

9. Tausif, Jayamanti Pandit, Divya Vohora, Mohd Aqil, Asgar Ali, and Yasmin Sultana. 2015.'Optimization of nanostructured lipid carriers of lamotrigine for brain delivery: in vitro characterization and in vivo efficacy in epilepsy', Expert opinion on drug delivery, 12(1): 81-94.

10. Haque, Shadabul, Shadab Md, Jasjeet Kaur Sahni, Javed Ali, and Sanjula Baboota. 2014. 'Development and evaluation of brain targeted intranasal alginate nanoparticles for treatment of depression', Journal of psychiatric research, 48(2) : 1-12.