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[1]Actimus Biosciences Private Limited, 3rd and 4th ÀRRU 9DUXQ 7RZHUV .DVWXUED 0DUJ 6LULSXUDP 9LVDNKDSDWQDP $QGK
[2]'HSDUWPHQW RI 3KDUPDFHXWLFDO $QDO\VLV DQG 4XDOLW\ $VVXUDQFH %DSDWOD &ROOHJH RI 3KDUPD
[3]'HSDUWPHQW RI 3KDUPDFHXWLFDO 6FLHQFHV $ 8 &ROOHJH RI SKDUPDFHXWLFDO 6FLHQFHV $QGKUD 8Q

Abstract

$ SUHFLVH VHQVLWLYH OLTXLG FKURPDWRJUDSK\ WDQGHP PDVV VSHFWURP YDOLGDWHG IRU WKH TXDQWLWDWLYH GHWHUPLQDWLRQ RI 7UD]RGRQH LQ KXPDQ OLTXLG H[WUDFWLRQ &KURPDWRJUDSKLF VHSDUDWLRQ RI GUXJ ZDV DFKLHYHG E ZLWK LVRFUDWLF PRELOH SKDVH RI P0

$PPRQLXP $FHWDWH S+ 2UJDQLF PL[WXUH DW D ÀRZ UDWH RI PO RI DFHWRQLWULOH PHWKDQRO 4XHWLDSLQH ZDV XVHG DV LQWHUQDO VWD $3, WDQGHP PDVV VSHFWURPHWHU XVLQJ SRVLWLYH HOHFWUR VSUD\ LRQL]DW PHWKRG DW P] DQG ÃR ` 0 URPH\ € cp Ð 0ì• 00 <0R ZDV XVHGR U XU DW KLJK PLGGOH DQG ORZ TXDOLW\ FRQWURO VDPSOHV ZDV IRXQG WR EH IRU LQWHUQDO VWDQGDUG 7KH SURSRVHG PHWKRG ZDV IRXQG WR EH YDOLGDWH UHLQMHFWLRQ UHSURGXFLELOLW\ DQG VWDELOLW\ VWXG\

Keywords: Trazodone; Quetiapine; LC-MS/MS; Validation

Introduction

Trazodone is chemically 2-{3-[4-(3-chlorophenyl)piperazin-1-yl] propyl}-2H,3H-[1,2,4]triazolo[4,3-a]pyridin-3-one. It is a serotonin antagonist and reuptake inhibitor (SARI), which is a second generation antidepressant compound belonging to the class of phenylpiperazine. It acts as a serotonin agonist at high doses and low doses. e drug showing antidepressant activity is due to the blockage of serotonin reuptake by inhibiting serotonin reuptake pump at the presynaptic neuronal membrane. Trazodone shows its therapeutic actions through 5-HT_{2A} receptors. Trazodone also induces anti-anxiety and sleep-inducing e ects [1]. It does not have similar properties to selective serotonin reuptake inhibitors (SSRIs) since its inhibitory e ect on serotonin reuptake and 5-HT_{2C} receptors are relatively weak [2]. e result of alpha-adrenergic action blocking and modest histamine blockade at H_1 receptor due to sedative e ect of trazodone. It weakly blocks presynaptic alpha2-adrenergic receptors and strongly inhibits postsynaptic alpha1 receptors. Trazodone does not show any action on the reuptake of norepinephrine or dopamine within the CNS. It has fewer anticholinergic side e ects than most of the tricyclic antidepressants such as dry mouth, constipation and tachycardia. Trazodone metabolizes to its primary m-chlorophenyl piperazine (mCPP) which is a non selective serotonin receptor agonist which might outweigh the bene ts of Trazodone [3-6].

e o cial methods for the determination of trazodone in pharmaceutical dosage forms includes potentiometric non-aqueous titration with perchloric acid [7] and HPLC using an octadecyl silane column and methanol–0.01 M ammonium phosphate bu er pH 6.0 (60:40) as the mobile phase [8]. Several analytical methods that have been reported for the determination of Trazodone in pharmaceutical formulations such as spectrophotometry [9-12], ion-selective electrode [13], voltammetry [14,15], colorimetry [16], instrumental TLC [17] and HPLC [18-20]. Various methods have been reported for the determination of Trazodone in biological uids, including HPLC [21-25], capillary gas chromatography [26], GC-MS/MS [27] and LC-MS/MS [28]. A combination of spectrophotometric, spectro uorimetric and LC determination of Trazodone has been also reported [29]. In this paper the main objective of the study was to develop a sensitive, rapid, precise, accurate method of determining trazodone in human plasma without interference from its metabolic products having Limit of Quanti cation 10.001 ng/ml using liquid-liquid extraction. e structures of Trazodone and Quetiapine are displayed in gure 1.

Materials and Methods

Reagents and chemicals

Trazodone (99.00% purity), Quetiapine (99.56% purity) were obtained from Splendid Labs Pvt Ltd., Pune, India. Methanol of HPLC grade obtained from Merck, Mumbai India. Acetonitrile and Tertiary Butyl Methyl Ether (TBME) of HPLC grade, Ammonium Acetate and Ammonia of GR/AR grade were purchased from Fisher scienti c Pvt. Ltd., Mumbai, India. High purity water was prepared through a Milli-Q water puri cation system.

Instrumentation

LC-MS/MS analysis was performed using API 3200 triple quadrupole instrument (Applied Biosystems SCIEX, Toronto,

*Corresponding author: Ravi Prakash PVDLS, Actimus Biosciences Private /LPLWHG UG DQG WK ÀRRU 9DUXQ 7RZHUV .DVWXUE $QGKUD 3UDGHVK ,QGLD 7HO)D[
GUSYGOV#JPDLO FRP

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Citation: 5DYL 3UDNDVK 39'/6 6XPDGKXUL % 6ULNDQW 3/& 06 06 0HWKRG IRU 'HWHUPLQDWLRQ RI 7UD]RGR GRL VFLHQWL¿FUHSRUWV

Citation: 5DYL 3UDNDVK 39'/6 6XPDGKXUL % 6ULNDQWK 0

standards to give concentrations of Lower Limit of Quanti cation Quality Control (LLOQ QC) 10.610 ng/ml, Lower Quality Control (LQC) 25.690 ng/ml, Medium Quality Control (MQC) 1284.485 ng/ml and Higher Quality Control (HQC) 2177.092 ng/ml. e samples were stored at -70 ± 15°C for further processing.

Sample preparation

50 µl of internal standard solution (400 ng/ml) was added into labeled ria vial tubes and spiked with 300 µl of plasma sample (respective concentration) into each tube and vortexed brie y. 100 µl of 2.0% (v/v) Ammonia solution was added to the above ria vial and vortexed. To it 2.5 ml of the Tertiary Butyl Methyl Ether (TBME) solution was added and vortexed at 2000 rpm for about 10 minutes. en the samples were centrifuged at 4000 rpm for approximately 10 min at ambient temperature. e upper organic layer from each sample was transferred into pre-labeled auto sampler vials and was evaporated until dryness under the Nitrogen evaporator. en the samples were reconstituted with 0.3 ml of mobile phase and analyzed.

Data processing

e MRM chromatographic peaks were integrated using Analyst so ware version 1.5.1 supplied by MDS technologies. Peak area ratios of Trazodone to Quetiapine were plotted versus concentration and a linear curve t, weighted by 1/X2 (where X = concentration) was used to produce the regression line.

Results and Discussion

Method validation

e validation parameters such as linearity, precision, accuracy, recovery, reinjection reproducibility and stability studies were conducted according to USFDA guidelines [30].

Linearity

Calibration curves were linear over the concentration range 10.001-3036.634 ng/ml for Trazodone. e best linear t and least square residuals for the calibration curve could be achieved with the linear equation y=mx + c with a 1/x2 weighing factor, where y was the peak area ratio of Trazodone to Quetiapine and x was the concentration of Trazodone. e correlation coe cient(r) for Trazodone was above 0.9994 (Figure 5) over the concentration range.

Lower Limit of Quanti cation (LLOQ)

LLOQ, the lowest concentration in the standard curve, which can be measured with acceptable mean response for analyte peak at the assay sensitivity limit (10.001 ng/ml), was vefold greater than the mean response for the peak in blank human plasma samples at the retention time of the analyte (Figures 6 and 7).

Precision and accuracy

Intra and inter batch accuracy and precision evaluations were performed by repeated analysis of Trazodone in human plasma. e run consisted of a six replicates of each LLOQQC, LQC, MQC and HQC samples. e overall precision of the method expressed as relative standard deviation and accuracy of the method. Inter day batch accuracy ranged from 89.6% to 105.8% and precision ranged from 1.8% to 10.5%. Intraday batch accuracy ranged from 91.5% to 104.3% and Lcix5(i)-8(ac)-296ion (8(e)-13(cis3(c)4(w)v(e)5(r L3(c3)]TJ EMC /Span

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stability, wet extract stability (in refrigerator and on bench top) were