International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.11 No.11, pp 232-241, 2018

Development and validation of LC-MS/MS method for the estimation of Droxidopa in Human Plasma

Venkata Ramu Derangula[1, 2]*, Jyothi Thumma [3] and Venkateswarlu Ponneri[4]

[1]Research Studies, Rayalaseema University, Kurnool-518 002, India
[2]ACR Laboratories, Ramanthapur, Hyderabad–500 013, India
[3]Center for Pharmaceutical Sciences, Jawaharlal Nehru Technological University, Kukatpally, Hyderabad–500 085, India
[4]Analytical and Environmental Chemistry Division, Department of Chemistry, Sri Venkateswara University, Tirupati–517502, India

Abstract : A high performance liquid chromatography mass spectrometric method for the estimation of droxidopa in human plasma in positive ion mode was developed and validated using levodopa as internal standard (IS). Sample preparation was accomplished by solid-phase extraction technique. The eluted samples were chromatographed on Hypurity advance, 4.6*50mm, 5µm (Thermo Scientific) column using a mobile phase consisting of 0.1% formic acid and methanol (80:20, v/v).The method was validated over a concentration range of 5.009 ng/mL to 3020.500 ng/mL for droxidopa. This validation report provides the results of analyte matrix, selectivity, matrix effect, sensitivity determinations, calibration standards and quality control samples data, precision and accuracy data, the results of recovery, various stabilities, run size evaluation, concomitant drug effect and dilution integrity.
Key Words: Droxidopa, Solid Phase Extraction (SPE),Method Validation,LC-MS/MS.

Introduction

Droxidopa (l-threo-3,4-dihydroxyphenylserine) is an orally administered synthetic precursor amino acid converted both peripherally and centrally into norepinephrine.[1]NORTHERA is indicated for the treatment of orthostatic dizziness, lightheadedness, or the "feeling that you are about to black out" in adult patients with symptomatic neurogenic orthostatic hypotension (nOH) caused by primary autonomic failure (Parkinson's disease [PD], multiple system atrophy, and pure autonomic failure), dopamine beta-hydroxylase deficiency, and non-diabetic autonomic neuropathy.[2]

Although droxidopa is widely used in clinical practice, the report method concerning the quantitation of droxidopa in human plasma is scanty. The most widely used method is a high-performance liquid chromatography (HPLC) with electrochemical detection [1,3,4] but this method needs a complex alumina extraction process and consumed large volume of biological samples. Jeon et al.[5] determinate droxidopa in rat

DOI= http://dx.doi.org/10.20902/IJCTR.2018.111124

serum by HPLC with fluorescence detection and this method was time-consuming for the fluorescence derivatization process. Gupta et al.[6] developed a novel electrochemical sensor based on ZnO nanoparticle and ionic liquid binder for square wave voltammetric to determine droxidopa in pharmaceutical and urine samples. Tajik et al.[7] determined droxidopa and carbidopa using a carbon nanotubes paste electrode. However, these two methods are not suitable for pharmacokinetic study for the cumbersome preparation of the sensor and electrode. In this paper, a new ultra performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) method was developed and validated to quantify droxidopa in human plasma. This method consumed only 100 L human plasma, using a simple protein precipitation extraction method as a high-throughput tool for pharmacokinetic study.

An efficient bioanalytical method should rapid, simple and consume less sample volume for analysis. Also, it should be specific and selective to avoid possible inferences at mass transition of analyte and the internal standard. [8-28]The reported methods were suffering from lack of sensitivity, use of more sample volume, longer chromatographic run time and employs non-deuterated compounds as internal standards which may results poor precision and accuracy values, where compensation for matrix effect is not possible.

Materials and Method

Materials

Hydrochloric acid, boric acid and acetone were procured from Fischer Scientific. Citric acid, sodium meta bisulphate and formic acid (AR grade) were procured from Merck Limited. Methanol (HPLC grade) was procured from JT Baker. Hypurity advance, 4.6*50mm, 5µm was procured from Thermo Scientific. Working standard of droxidopa (purity- 99.58%) was received from Clearsynth Labs Limited. Internal standard levopdopa (purity-99.76%) was supplied by VIVAN Life Sciences Pvt. Limited.

Instrumentation

Analytical separation was achieved on Liquid chromatography (Shimadzu Prominence)coupled withtandem mass spectrometer (AB SCIEX API 4500) and the system was operated using software Analyst 1.6.2.Chromatographic separation was achieved using a mobile phase consisting of binary solution of 0.1% formic acid and methanol in the ratio of 80:20 %v/v on a reverse phase Hypurity advance, 4.6*50 mm, 5 µm (ThermoScientific) column with a flow rate of 1.0 mL/min. Sample cooler temperature was maintained at 10°C, injection volume was 15 µL and total run time was 2.0 min.

The mass spectrometer was operated in the positive ion mode using electrospray ionization. The MS/MS spectra of droxidopa and levodopa produced two fragments each, called parent and product. The two fragments of droxidopa were at *m*/*z* 214.300 and 152.100 respectively and that of Levodopa were at 198.200 and 152.100 respectively. The electrospray parameters used were an Ion spray voltage of 5500V, using temperature of 600 °C with curtain gas and collision gas flowing at 35 and 5 psi respectively. The collision energy was 12 V with both collision cell exit potential (CXP) and entrance potential (EP) maintained at 10 V. Dwell time was 200 ms.

Droxidopa stock solution (1 mg/mL)

Weighed about 5 mg of droxidopa working standard and transferred to a 5 mL clean glass volumetric flask, dissolved in 3N hydrochloric acid and made up the volume with the same to produce a solution of 1 mg/mL. Corrected the above concentration of droxidopa solution accounting for its potency and the actual amount weighed.

The stock solutions were diluted to suitable concentrations using a mixture of HPLC grade methanol and HPLC grade water (diluent) in the ratio of (40:60 v/v) for spiking into plasma to obtain calibration curve (CC) standards and quality control (QC) samples. For the preparation of calibration curve standards and quality control samples two separate stock solutions were prepared and used. All other final dilutions (system suitability dilutions, recovery aqueous mixture, etc.) were prepared in reconstitution solution. The CC and QC samples were prepared from two separate independently weighed stocks.

Levodopa stock solution (Internal standard)

Weighed about 2 mg of Levodopa transferred to a 2 mL volumetric flask, dissolved in 3N hydrochloric acid and made up the volume with the same to produce a solution of 1 mg/mL. The stock solution was diluted to suitable concentration (10 µg/mL) using diluent for internal standard dilution.

Calibration curve standards and quality control samples

Calibration curve standard consisting of a set of ten non-zero concentrations ranging from 5.009 ng/mL to 3020.500 ng/mL of droxidopa were prepared. Prepared quality control samples consisted of concentrations of 5.030 ng/mL (LLOQ QC), 15.105 ng/mL (LQC), 377.624 ng/mL (MQC1), 1510.497 ng/mL (MQC2), and 2271.425 ng/mL (HQC) for droxidopa. These samples were stored at –70±10°C until use. Twelve sets each of quality control samples for dilution integrity were prepared by spiking about 1.60 times (4845.706 ng/mL) and 3.21 times (9691.412 ng/mL) highest standard concentration of droxidopa. From these, six sets each of two times dilution and four-time dilution was performed.

Note: 5% of 0.2M citric acid buffer shall be add to the blank plasma (Add 50µL of 0.2M citric acid buffer to the 950µL of plasma)

Sample preparation

The samples were thawed at room temperature and vortexed to ensure complete mixing of the contents. 250 µL of the plasma sample was transferred into pre-labelled RIA vial tubes, added 15 µL 20mM sodium metabisulphate solution and 25 µL of internal standard dilution (10µg/mL of levodopa) and vortexed. Then 500 µL of water was added and vortexed.

The sample mixture was loaded onto Orochem Alumina Basic 100 mg/1ml, cartridges that were pre-conditioned with 1.0 mL of HPLC grade methanol followed by 1.0 mL of 0.5M boric acid solution. After applying the maximum pressure, the extraction cartridge was washed with 1 mL of 50% acetone solution in water followed by 1.0 mL of 0.01N hydrochloric acid solution and 1.0 mL of methanol. Then the samples were eluted with 1.0 mL of a mixture of formic acid, methanol and water (3:20:77, v/v/v).

Results

Method development

Source and compound dependent parameters were optimized by infusing (5µL/min) the 100 ng/mL concentration of analyte and the IS separately. High intense peaks were found in positive mode than the negative mode. Protonated form of analyte and IS, $[M+H]^+$ ion was the parent ion in the Q_1 spectrum and was used as the precursor ion to obtain Q_3 product ion spectra. The most sensitive mass transition was observed from m/z 214.3 to 152.1 for droxidopa and from m/z 198.2 to 152.1 for the IS. The dwell time for each transition was 200 ms.

Mobile phase composition was optimized with acetonitrile and methanol in combination with acidic buffers ammonium formate and ammonium acetate and volatile acids namely formic acid and acetic acid. Also, a variety of C_{18} columns were tested to get symmetric peak shape with short retention time.

Among different mobile phase combinations tested, 0.1% formic acid and methanol (80:20, v/v) gives symmetric peak shape and reproducible response for the analyte. Best chromatography results were obtained with Hypurity advance (50 x 4.6 mm, 5µm). The run time was set at 2 min with retention time of 0.7 min for droxidopa and the IS. Solid phase extraction (SPE) with Orochem alumina basic (100 mg/1mL) cartridges were used to extract the analyte from plasma, which gives neat and clear extract with minimal or no matrix effect. Levodopa was used as internal standard which exerts similar extraction, ionization and retention time with the analyte.

Selectivity

Representative chromatograms of extracted blank plasma and blank plasma spiked with the IS are given in Fig. 1 & 2 there was no significant interference from endogenous components observed at the mass transitions of droxidopa and internal standard. No interference was observed at retention time of levodopa when ULOQ concentration of droxidopa injected. Similarly, no significant interference was observed at retention time of droxidopa when working concentration of levodopa injected. A representative chromatogram LLOQ samples was displayed in Fig. 3.

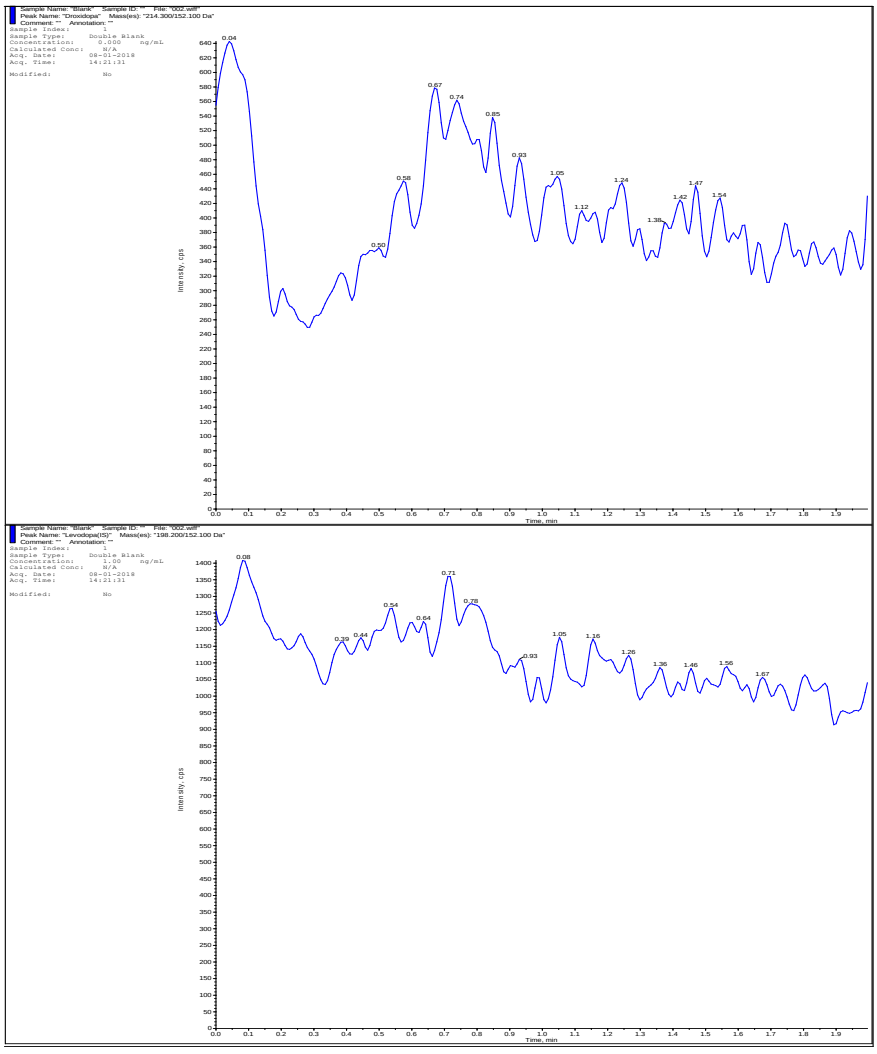

Figure 1: A representative chromatogram of blank plasma sample of droxidopa

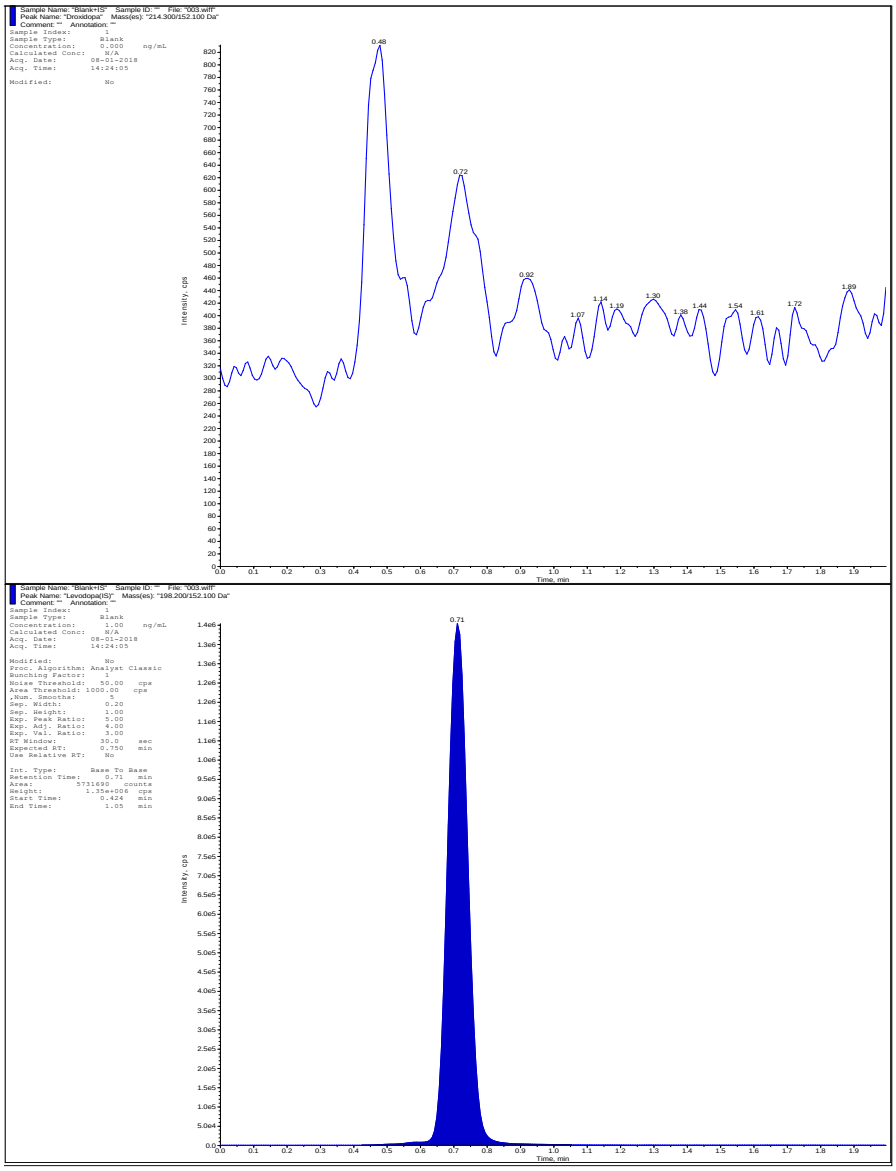

Figure 2: A representative chromatogram of blank plasma with internal standard sample of droxidopa

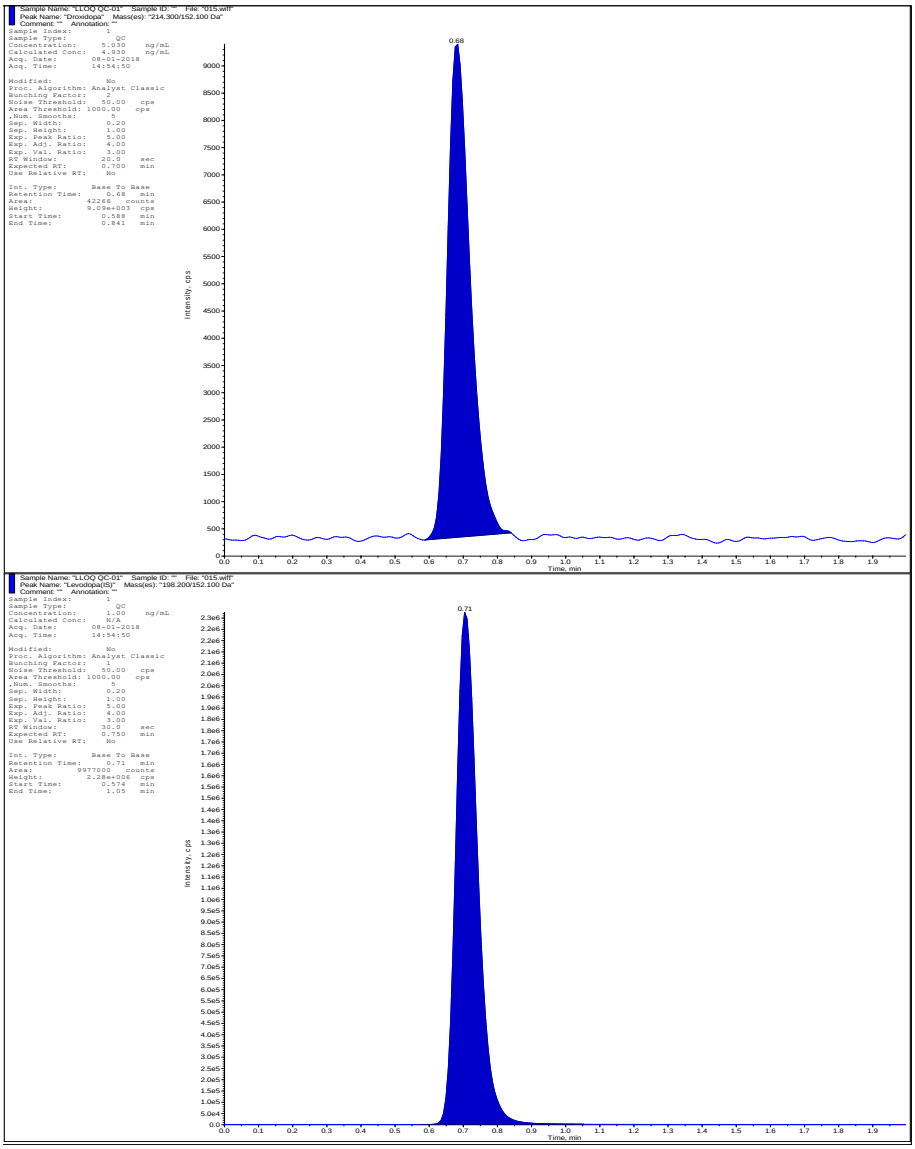

Figure 3: A representative chromatogram of LLOQ QC sample of droxidopa

Sensitivity

The lowest limit of reliable quantification for droxidopa in human plasma was set at the concentration of the LLOQ 5.009 ng/mL. The precision and accuracy for droxidopa at this concentration was found to be 2.82% and 96.32%, respectively.

Matrix effect

No significant matrix effect was observed in all the eight batches for droxidopa at low (LQC) and high (HQC) concentrations. The precision for IS normalized matrix factor at LQC and HQC level was found to be 2.74% and 2.51%, respectively and IS normalized factor was 1.101 for LQC and 1.059 for HQC.

Linearity

A regression equation with a weighting factor of 1/(concentration ratio)^2 of drug to IS concentration was judged to produce the best fit for the concentration-detector response relationship for droxidopa in human plasma. Correlation coefficient (r) was greater than 0.99 in the concentration range of 5.009 ng/mL to 3020.500 ng/mL for Droxidopa.

Precision and accuracy

The precision of the assay was measured by the percent coefficient of variation over the concentrations of LLOQ QC, LQC, MQC1, MQC2, and HQC samples during the course of validation. The accuracy of the assay was defined as the absolute value of the ratio of the calculated mean values of the LLOQ, low, middle and high quality control samples to their respective nominal values, expressed in percentage. The precision and accuracy data was given in Table 1.

Table 1: Precision and accuracy data for droxidopa.

Quality Control Run	Concentration found Mean±SD(ng/mL)	Precision (%)	Accuracy (%)
Intra-day variations (n=12 at each concentration)			
LLOQ	4.9 ± 0.13	2.66%	97.50%
LQC	15.41 ± 0.40	2.59%	102.05%
MQC1	380.69 ± 13.06	3.43%	100.81%
MQC2	1524.64 ± 22.39	1.47%	100.94%
HQC	2215.49 ± 51.63	2.33%	97.54%
Inter-day variations (n=36 at each concentration)			
LLOQ	5.16 ± 0.29	5.66%	102.49%
LQC	14.98 ± 0.89	5.97%	99.17%
MQC1	382.16 ± 16.98	4.44%	101.20%
MQC2	1516.60 ± 36.86	2.43%	100.40%
HQC	2244.15 ± 59.93	2.67%	98.80%
Nominal concentrations of LLOQ, LQC, MQC1, MQC2 and HQC are 5.03, 15.11, 377.62, 1510.50 and 2271.43 ng/mL, respectively			

Recovery

Six sets of aqueous (non-extracted) samples of LQC, MQC2 and HQC were prepared and injected along with extracted samples. The extracted samples (LQC, MQC2 and HQC) of droxidopa were compared with the non-extracted samples of LQC, MQC2 and HQC. The internal standard response in extracted samples of LQC, MQC2 and HQC were compared with the response of internal standard in the entire non-extracted eighteen quality control sample (LQC, MQC2 and HQC). The mean overall recovery of droxidopa was 48.17% with a precision range of 1.51% to 7.23%. The mean recovery of internal standard levodopa was 44.32% with a precision ranging from 3.27% to 4.30%.

Dilution integrity

12 sets of dilution integrity samples were prepared by spiking 1.60 times (4845.706 ng/mL) and another 12 sets of dilution integrity samples were prepared by spiking 3.21 times (9691.412 ng/mL) of highest standard concentration. Six sets of dilution integrity samples were processed by diluting them twice and four times. These quality control samples were analyzed along with a processed calibration curve standards (undiluted) of concentration range equivalent to that used for the calculation of precision and accuracy. The quality control sample concentrations were calculated using appropriate dilution factor. The results demonstrate acceptable dilution integrity for two and four time's dilution. A precision and accuracy results of droxidopa, for a dilution factor of 2 was 2.76% and 99.56%, respectively. Similarly, for a dilution factor of 4 was 2.53% and 99.46%, respectively.

Ruggedness

One precision and accuracy batch were processed by the different analyst and analyzed using different column of same make, different set of solutions. Within batch precision for LLOQ QC, LQC, MQC1, MQC2, and HQC was 4.10%, 2.20%, 1.16%, 1.74%, and 1.32%, respectively. Within batch accuracy for LLOQ QC, LQC, MQC1, MQC2, and HQC was 106.48%, 100.10%, 100.40%, 102.04%, and 101.42%, respectively.

Stabilities

The mean % nominal values were found to be within ±15% of the predicted concentrations for the analyte at their LQC and HQC levels and the precision (% CV) values were within 15% (Table 2) for all the stability tests carried out during the entire course of method validation. All the above stability results were found to be within the acceptable limits during the entire validation.

Table 2: Stability data for droxidopa.

Stability test	QC (spiked concentration ng/mL)	Mean ± SD(ng/mL)	Precision (%)	Accuracy/ Stability (%)
Process[a]	15.11	15.56 ± 0.31	2.01	103.00%
	2271.43	2326.05 ± 11.99	0.52	102.41%
Process[b]	15.11	15.72 ± 0.36	0.36	101.31%
	2271.43	2337.70 ± 22.47	0.96	99.46%
Bench top[c]	15.11	15.60 ± 0.77	4.96	103.29%
	2271.43	2326.83 ± 36.49	1.57	102.44%
Freeze thaw[d]	15.11	15.41 ± 0.25	1.59	102.01%
	2271.43	2313.55 ± 44.20	1.91	101.85%
Reinjection[e]	15.11	14.64 ± 0.51	3.46	96.91%
	2271.43	2107.43 ± 28.09	1.33	92.78%
Long-term[f]	15.11	15.43 ± 0.39	2.52	102.11%
	2271.43	2326.02 ± 19.89	0.86	96.81%
a: after 53 h 55 min in auto sampler at 10°C; b:after 5 days 17 h at -20°C; c: after 7 h 26 min at room temperature; d: after 3 freeze and thaw cycles; e: after 48 h 56 min of reinjection; f: at –70°C for 45 days long term stability.				

Concomitant drug effect

Concomitant drug effect was investigated to ensure the precision and accuracy is not compromised with potentially interfering concomitant medication. The effect of OTC drugs (Pantoprazole, paracetamol, ibuprofen, diphenhydramine, caffeine, dicyclomine and nicotine)on droxidopa analysis was tested. Six replicates of LLOQ QC, LQC and HQC samples were prepared in screened plasma spiked with concomitant drugs. Above quality control samples were processed and analyzed along with calibration curve.Within batch precision for LLOQ QC, LQC and HQC 4.42%, 10.03% and 1.91%, respectively. Within batch accuracy for LLOQ QC, LQC and HQC was 111.58%, 97.07% and 96.83%, respectively.

Conclusion

A simple and rapid LC–MS/MS assay method was developed and validated for the determination of droxidopa in human plasma. This method was fully validated as per US FDA guidelines and is well suitable for pharmacokinetic or bioavailability/bioequivalence application. The proposed SPE method gave consistent and reproducible recovery for the analyte. The total chromatographic runt time was set at 2 min, which is rapid and can analyze more number of samples in a day. The method can be applied for pharmacokinetic or bioavailability/bioequivalence studies.

References

1. Kaufmann H, Saadia D, Voustianiouk A, Goldstein DS, Holmes C, Yahr MD, Nardin R, Freeman R. Norepinephrine precursor therapy in neurogenic orthostatic hypotension, Circulation 108 (2003) 724–728.
2. Takagi H, Harima A. Analgesic effect of l-threo-3,4-dihydroxyphenylserine (l-DOPS) in patients with chronic pain, Eur. Neuropsychopharm. 6 (1996) 43–47.

3. Holmes C, Eisenhofer G, Goldstein DS. Improved assay for plasma dihydroxyphenylacetic acid and other catechols using high-performance liquid chromatography with electrochemical detection, J. Chromatogr. B 653 (1994) 131–138.
4. Goldstein DS, Holmes C, Kaufmann H, Freeman R. Clinical pharmacokinetics of the norepinephrine precursor l-threo-DOPS in primary chronic autonomic failure, Clin. Auton. Res. 14 (2004) 363–368.
5. Jeon HK, Nohta H, Ohtsubo K, Ohkura Y. Determination of z-threo-3,4-dihydroxyphenylserine and l-a-methyldopa in rat serum by high performance liquid chromatography with fluorescence detection, Anal. Sci. 5 (1989) 663–666.
6. Gupta VK, Sadeghi R, Karimi F. A novel electrochemical sensor based on ZnO nanoparticle and ionic liquid binder for square wave voltammetric determination of droxidopa in pharmaceutical and urine samples, Sensor. Actuat. B Chem. 186 (2013) 603–609.
7. Tajik S, Taher MA, Beitollahi H. Simultaneous determination of droxidopa and carbidopa using a carbon nanotubes paste electrode, Sensor. Actuat. B Chem. 188 (2013) 923–930.
8. Matta MK, Pilli NR, Jillela VLNSR. A validated liquid chromatography and tandem mass spectrometric method for simultaneous quantitation of tenofovir, emtricitabine and efavirenz in human plasma and its pharmacokinetic application. Acta Chromatogr, 2015; 27: 27-39.
9. Putluru SP, Matta MK, Ahire D, Subramanian M, Sinz M, Mandlekar S. A novel liquid chromatography tandem mass spectrometry method for the estimation of bilirubin glucuronides and its application to in vitro enzyme assays. Drug Metab Lett, 2016; 10(4): 264-269. 11.
10. Grundy JS, Kherani R, Foster RT. Sensitive high-performance liquid chromatographic assay for nifedipine in human plasma utilizing ultraviolet detection. B Biomed Appl, 1994; 654(1): 146-151. 12.
11. Murali MK, Ashok C, Adarsh G, Sharron S, Lin Xu, Katherine S, Vikram P, Rodney R. LC-MS/MS based quantitation of ciprofloxacin and its application to antimicrobial resistance study in Balb/c mouse plasma, urine, bladder and kidneys. DOI: 10.1039/C7AY02923C.
12. El-Sayed YM, Niazy EM, Khidr SH. High-performance liquid chromatographic method for thedetermination of nifedipine in plasma and its use in pharmacokinetic studies. J Clin Pharm Therap, 1993; 18, 325-330.
13. Roosemalen MC, Soons PA, Funaki T, Breimer DD, High-performance liquid chromatographic determination of the polar metabolites of nifedipine in plasma, blood and urine. J Chromatogr, 1991; 565(1-2): 516-522.
14. Janjanam KC, Bimireddy BPK, Kovvasu SPR, Ratna JV. Bioanalysis of darunavir in human plasma using liquid chromatography coupled with tandem mass spectrometry. Int J PharmTech Res, 2018; 11(1): 28-34.
15. Shah VP, Midha KK, Dighe S, McGilveray IJ, Skelly JP, Yacobi A, Layloff T, Viswanathan CT, Cook CE, Mcdowall RD, Pittman KA, Spector S. Analytical methods validation: bioavailability, bioequivalence and pharmacokinetic studies. Conference report. Eur J Drug Metab Pharmacokinet, 1991; 16: 249-255.
16. Matta MK, Pilli NR, Inamadugu JK, Burugula L, Jillela VLNSR. Simultaneous quantification of lamivudine, zidovudine and nevirapine in human plasma by liquid chromatography – tandem mass spectrometry and its application to a pharmacokinetic study. Acta Pharm Sin B, 2012; 2(5): 472-480.
17. Sadanaga T, Hikida K, Tameto K, Matsushima Y, Ohkura Y. Determination of nifedipine in plasma by high-performance liquid chromatography. Chem Pharm Bull, 1982; 30(10): 3807-3809.
18. Kovvasu SPR, Yeung S, Kunamaneni P, Kodali B. Bioanalysis of febuxostat in human plasma by liquid chromatography/tandem mass spectrometry. Int J PharmTech Res, 2018; 11(2): 168-176.
19. Kleinbloesem CH, Van Harten J, Van Brummelen P, Breimer DD. Liquid chromatographic determination of nifedipine in plasma and of its main metabolite in urine. J Chromatogr, 1984; 308: 209-216.
20. Adireddy V, Kovvasu SPR, Ravella VN. Simple and rapid LC-MS/MS method for the determination of rasagiline in human plasma. Eur J Biomed and Pharmaceu Sci, 2017; 4(11): 695-699.
21. Matta MK, Burugula L, Pilli NR, Inamadugu JK, Jillela VLNSR. A novel LC-MS/MS method for simultaneous quantification of tenofovir and lamivudine in human plasma and its application to a pharmacokinetic study. Biomed Chromatogr, 2012; 26(10): 1202-1209.
22. Kovvasu SPR, Adireddy V, Janjanam KC. Novel LC-MS/MS method for the determination of celecoxib in human plasma. Int J ChemTech Res, 2018; 11(03): 303-311.
23. Neelima B, Pigili RK, Matta MK, Bindu VH, Yejella RP, Simultaneous estimation of simvastatin and ezetimibe by RP-HPLC in pure and pharmaceutical dosage form. Oriental J Chem, 2008; 24(1): 195-200.

24. Soons PA, Schellens JH, Roosemalen MC, Breimer DD. Analysis of nifedipine and its pyridine metabolite dehydronifedipine in blood and plasma: review and improved high performance liquid chromatographic methodology. J Pharm Biomed Anal, 1991; 9(6): 475-484.
25. Kodali B, Kovvasu SPR, Kunamaneni P, Janjanam KC, Pappula NR. Bioanalytical method for quantitative determination of olmesartan in human plasma by liquid chromatography-tandem mass spectrometry. World J Pharm Pharmaceu Sci, 2018; 7(4): 946-957.
26. Diaz MT, Kelly MT, Hua C, Smyth MR. High-performance liquid chromatographic determination of nifedipine, nicardipine and pindolol using a carbon fibre flow-through amperometric detector. J Pharm Biomed Anal, 1991; 9(10-12): 889-893.
27. Gandla K, Repudi L, Kovvasu SPR, Rao RN. Simple and rapid determination of rosuvastatin in human plasma by LC-MS/MS. World J Pharm Pharmaceu Sci, 2017; 6(11): 1027-1037.
28. Kovvasu SPR, Kunamaneni P, Yeung S, Kodali B. Determination of colchicine in human plasma by a sensitive LC-MS/MS assay. World J Pharm Pharmaceu Sci, 2018; 7(3): 35-44.

*****