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FAST AND SENSITIVE VALIDATED METHOD FOR QUANTITATION OF HALOPERIDOL IN HUMAN PLASMA USING ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY (UPLC) WITH DIODE ARRAY DETECTION

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ABSTRACT

A simple, sensitive and rapid ultra-performance liquid chromatography (UPLC) method has been developed and validated for estimation of haloperidol (HAL) in human plasma in vitro. Sample was prepared by simple liquid-liquid extraction technique. Chromatographic separation of HAL and IS was achieved by using Acquity UPLC BEH shield RP18 column and maintained at 35ºC temperature. The mobile phase consist of a mixture of 12 mM ammonium acetate buffer pH 3.5 adjusted with acetic acid (80%, v/v) and acetonitrile (20%, v/v) at a flow rate of 0.4 mL/min. Quantification was carried out on a photodiode array detector set at 240 nm. HAL and IS retention time were found to be 1.8 and 1.4 respectively. The total run time was 2.5 min. The method was validated for specificity, selectivity, recovery, accuracy, precision, recovery and stability. The calibration curve was linear the concentration range of 1 to 100 ng/mL. The method can be employed in therapeutic drug monitoring practices and clinical toxicological assays.

KEYWORDS: UPLC; Haloperidol; Therapeutic drug monitoring; human plasma.

INTRODUCTION

Antipsychotic agents are used to manage the psychotic episodes and behavioural symptoms in psychiatric patients. First generation Antipsychotics (haloperidol and chlorpromazine) are popular for the treatment of schizophrenia.[1] Haloperidol (HAL) is a known dopamine antagonist and has been widely used in the treatment of psychosis.[2] In addition, the drug has established that the notable value for determining poor compliance of patients in the Therapeutic Drug Monitoring (TDM) studies. Therefore, highly selective, sensitive and accurate bio-analytical methods are essential since most of these drugs are presents in low concentration in plasma.[3]

Several analytical methods have been described for determination of antipsychotics using HPLC; hence these methods have various disadvantages in terms of expensive solvent, complex sample extraction and run time. In previous studies, different High Performance Liquid Chromatography (HPLC) methods with UV detection[4,5], caplillary gas-liquid chromatography[6], coulometric[7,8] and fluorescence detection[9,10] were reported to detect and quantify the antipsychotic medications. Recently numerous studies have developed methods base on Liquid chromatography coupled with mass spectrometry (LC-MS/MS) to have increased sensitivity in detection with less run time.[11,12] Although LC-MS/MS methods are beneficial in many ways, the costly instrumentation and less accessibility to regular hospital practice and laboratories are major limitations.

The aim of the study is to establish a rapid, simple and accurate UPLC method to detect and quantify the HAL in plasma. In this view, a simple, sensitive and reproducible Ultra High Performance Liquid Chromatography (UPLC) with Diode Array Detection (DAD) for HAL was developed and validated. The method has less run time, can able to transfer to LC-MS/MS if needed as compatible buffer and chromatographic conditions were used in determination. It can be employed in therapeutic drug monitoring and clinical toxicological studies.

MATERIALS AND METHODS

Chemicals and reagents

Haloperidol (HAL) and clozapine (IS) were purchased from Sigma-Aldrich, India. Acetic acid and Methyl tert-butyl ether of HPLC grade were obtained from Himedia, India. The HPLC grade acetonitrile and ammonium acetate were purchased from Sigma-Aldrich, India.

Human blank plasma was obtained from blood bank at PSG Hospitals, Coimbatore. Ultrapure water was obtained using a milli-Q system from Millipore (Milford, USA).

Instrumentation and Chromatography conditions

Analysis was performed on an UPLC system that consisted of a Waters Acquity H class UPLC system equipped with a quaternary pump and 96-vial autosampler coupled with a diode array UV detector (Waters, Milford, MA, USA) set at 240 nm. The analysis was carried out on an Acquity UPLC BEH C18 column from Waters (2.1 mm × 100mm; 1.7µm) and the column temperature maintained at 35ºC and the autosampler was kept at 8ºC. The mobile phase consisted of a mixture of 12 mM ammonium acetate buffer pH 3.5 adjusted with acetic acid (80%, v/v) and acetonitrile (20%, v/v) at a flow rate of 0.4 mL/min. The total run time was 2.5 min and the injection volume was 5µL.

Preparation of stock and standard solution

Stock solution of HAL and IS were prepared at a concentration of 1 mg/mL. The stock solution of HAL and IS were stored at 4ºC and were found to be stable for one month. Further dilution was made in methanol: water (50:50. v/v) to produce working stock solution for the calibration standards and quality control (QC) standards. The IS working stock solution of 250 µg/mL was prepared in methanol-water (50:50 v/v) by dilution of the stock solutions. Calibration samples were prepared by spiking 240 µL of blank human plasma with the appropriate working solution of HAL (10µL) on the day of analysis. All the samples were stored at -80 ± 10°C until analysis.

Sample preparation

A liquid-liquid extraction method was carried out for extraction of HAL from human plasma. To an aliquot of 250µL of plasma, IS solution (25µL of 5µg/mL) and 12mM ammonium acetate (pH-9.0) were added and mixed for 30 seconds on a spinix vortex shaker (Tarsons, India). After the addition of 3.0 mL of tert-butyl methyl ether, the mixture was vortexed for 5 min and followed by centrifuged at 10,000 rpm for 5 min at 4ºC on an eppendrof 5810R centrifuge (Eppendrof AG, Hamburg, Germany). The clear organic layer (2.5mL) was separated into 5mL polypropylene tubes and evaporated to dryness at 40ºC using nitrogen evaporator (Turebovap®, Biotage, USA). The residue was reconstituted in 200µL of the mobile phase, vortex mixed for 3.0 min and 5µL were injected onto the UPLC system for analysis.

METHOD VALIDATION

The present method was validated for specificity, linearity, accuracy, precision, recovery and stability using Food and Drug Administration (FDA) guidelines[13] for the assay in human plasma.

Specificity

Specificity of the method was determination by analysing six replicates of blank plasma obtained from six different donors and investigating the possible interference at the LC peak region for HAL and IS. The acceptance criterion was that at least five out of six lots should have response less than five times the lower limit of quantitation (LLOQ) level in the same matrix.

Linearity

Linearity was assessed by linear regression analysis with the use of working standard solutions and spiked biological samples containing the drugs of interest at different concentrations within the range of 1.0 to 100 ng/mL. The calibration curve were constructing by plotting the ratio of the peak area of each analyte to the peak area of the IS versus the normal drug concentration. The slopes, intercept and coefficients of determination were calculated with least square linear regression analysis of the data with the use of a $1/x^2$ (x-concentration) weighting factor. The acceptance criteria for each back calculated standard concentration were ±15% deviation from the nominal value except at LLOQ which was set at ±20%.[13]

Accuracy and precision

Accuracy and precision for intra- and inter-day assay samples were estimated by analysing six replicates containing HAL at three different QC levels, that is, 5, 50 and 85ng/mL. Accuracy, which represents the closeness of agreement between the mean values obtained from the series of measurements by the method to the actual value. The accuracy should be within 85-115% of the nominal value, except at LLOQ, where it should not deviate more than 20 %.[13]

Recovery

The efficiency of HAL and IS extraction from the human plasma was determined by comparing the responses of the analyte extracted from replicate QC samples (n=6) with the response of analyte from the neat standards at equivalent concentration by a liquid-liquid

extraction process. HAL recovery was assessed by analytes of the three various concentration (5, 50, 85 ng/mL) while the recovery of the IS was determined at a single concentration of 2 µg/mL.

Stability

Stability assessment was conducted to evaluate the stability of HAL in human plasma samples under different conditions. Freeze-thaw (three cycles) and long-term stability was assessed by QC samples stored at 30 days at -80ºC. Bench-top stability study evaluate the stability of analyte in plasma at room temperature was determined at low (5 ng/mL) and high (85 ng/mL) QC concentration for 8 h.

RESULTS AND DISCUSSION

Specificity

Specificity of the developed method was determined by comparing the chromatogram of blank plasma, blank plasma spiked with IS and with analyte (1ng/mL). As shown in Fig. 1 and Fig. 2, no interfering peaks from endogenous compounds were observed at the retention time of analyte and IS. The total chromatographic run time was 2.5 min.

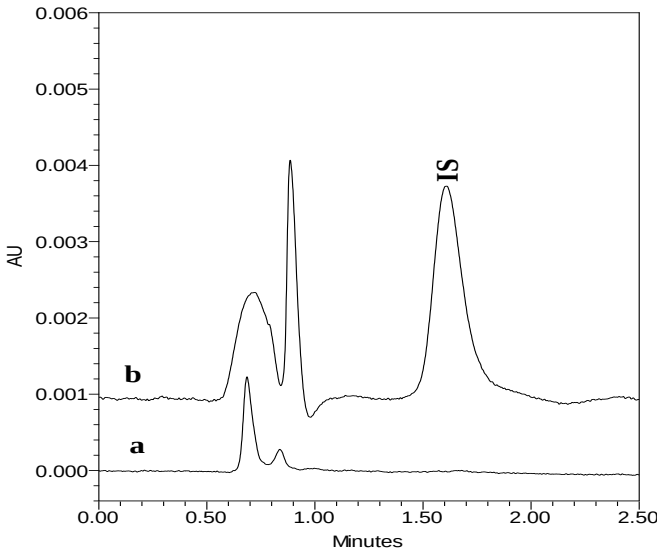

Fig. 1. Representative UPLC chromatograms of (a) blank plasma sample; (b) blank plasma sample spiked with IS.

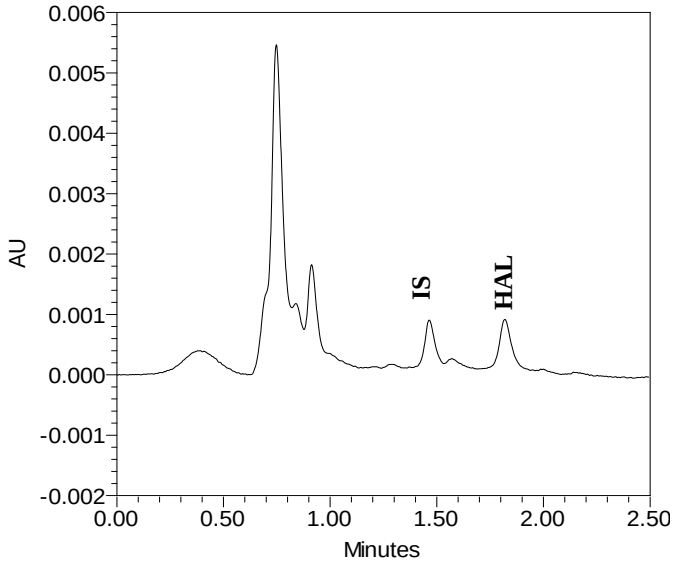

Fig. 2. Representative UPLC chromatograms of blank plasma spiked with analyte (1ng/mL).

Linearity

The quantification of HAL was based on the area ratio of the analyte over the IS vs HAL concentration. The plasma calibration curve was constructed using eight calibration standards (viz., 1-100 ng/mL) in human plasma and showed excellent linearity with regression coefficient ($r^2 > 0.991$). The standard curve had a consistent reproducibility over the standard concentration across the calibration range. The calibration curve was prepared by determining the best fit of peak-area ratio (peak area analyte / peak area IS) vs concentration and fitted to the y=mx+c using weighting factor ($1/x^2$). The lowest concentration with the RSD < 20% was taken as the LLOQ and was found to be 1.0 ng/mL for HAL. The percentage accuracy observed for the mean of back-calculated concentration for four calibration curves for the HAL was within 92.72-106.57.

Accuracy and precision

Accuracy and precision data for intra- and inter-day human plasma samples are presented in Table 1. The assay values on both the occurrences (intra- and inter-day) were found to be within the accepted variable limits.

Table 1. Intra- and inter-day precision and accuracy of HAL in human plasma *in vitro*.

Nominal concentration (ng/mL)	Intra-day (n = 6)			Inter-day (n = 18)		
	Measured concentration (mean ± SD),	CV (%)	Accuracy (%)	Measured concentration (mean ± SD),	CV (%)	Accuracy (%)
HAL						
4.94	4.85 ± 0.13	2.68	98.18	4.82 ± 0.14	2.90	97.57
50.16	48.76 ± 0.67	1.37	97.21	49.34 ± 1.37	2.78	98.37
84.92	84.55 ± 2.69	3.18	99.56	83.80 ± 2.22	2.65	98.68

Recovery

Liquid-liquid extraction method gave adequate recovery and cleaner samples. Comparison of neat standards vs human plasma-extracted standards were estimated for HAL (5, 50, 85 ng/mL) and the mean recovery was ranged from 81.51 ± 3.51, 78.91 ± 1.70 and 81.18 ± 1.86%, respectively. The recovery of IS at 2 µg/mL was 77.82 ± 1.61%.

Stability

The expected concentration of HAL at 5 and 85 ng/mL samples deviated within ±15% of the nominal concentrations in a series of stability test, viz. Bench-top (8 h), three freeze-thaw cycles and long-term stability at -80ºC for 30 days (Table 2). The results were found to be within the assay variability limits during the entire process.

Table 2. Stability of HAL in human plasma *in vitro* under different conditions (n=6).

Nominal concentration (ng/mL)	Bench –top (room temperature for 8 h)		Autosampler (8°C for 26 h)		Freeze-thaw cycles		Long-term stability (-80°C for 30 days)	
	Measured concentration (mean ± SD, ng/mL)	Bias (%)	Measured concentration (mean ± SD, ng/mL)	Bias (%)	Measured concentration (mean ± SD, ng/mL)	Bias (%)	Measured concentration (mean ± SD, ng/mL)	Bias (%)
HAL								
4.94	4.89 ± 0.16	-1.02	4.91 ± 0.17	-0.61	4.95 ± 0.17	0.20	5.01 ± 0.12	1.40
84.92	85.05 ± 1.82	0.15	83.28 ± 1.99	-1.97	83.53 ± 0.56	-1.66	83.36 ± 1.91	-1.87

CONCLUSION

This paper describes a rapid, sensitive and specific UPLC-DAD method to quantify the HAL and its validation for the analysis of HAL in human plasma. The UPLC-DAD method has significant advantages over other techniques including the simplicity of shorter

chromatographic run time, sharper peaks and higher extraction recovery. It can be employed in therapeutic drug monitoring practices and clinical toxicological assays. This method provides similar sensitivity like LC-MS/MS and can be used as an alternative method with a highly cost effective and sensitive analytes yield.

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