Journal of Pharmaceutical and Biomedical Analysis 152 (2018) 215–223

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Selective and rapid determination of tadalafil and finasteride using solid phase extraction by high performance liquid chromatography and tandem mass spectrometry

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ARTICLE INFO

Article history:
Received 19 October 2017
Received in revised form 4 January 2018
Accepted 11 January 2018

Keywords:
Tadalafil
Finasteride
Human plasma
Solid phase extraction
Validation
Liquid chromatography–tandem mass spectrometry

ABSTRACT

Highly selective and fast liquid chromatography-tandem mass spectrometric (LC–MS/MS) method was developed and validated for simultaneous determination of tadalafil (TDL) and finasteride (FNS) in human plasma. The method was successfully applied for analysis of TDL and FNS samples in clinical study. The method was validated as per USFDA (United States Food and Drug Administration), EMA (European Medicines Agency), and ANVISA (Agência Nacional de Vigilância Sanitária-Brazil) bio analytical method validation guidelines. Glyburide (GLB) was used as common internal standard (ISTD) for both analytes. The selected multiple reaction monitoring (MRM) transitions for mass spectrometric analysis were *m/z* 390.2/268.2, *m/z* 373.3/305.4 and *m/z* 494.2/369.1 for TDL, FNS and ISTD respectively. The extraction of analytes and ISTD was accomplished by a simple solid phase extraction (SPE) procedure. Rapid analysis time was achieved on Zorbax Eclipse C18 column (50 × 4.6 mm, 5 μm). The calibration ranges for TDL and FNS were 5–800 ng/ml and 0.2–30 ng/ml respectively. The results of precision and accuracy, linearity, recovery and matrix effect of the method are acceptable. The accuracy was in the range of 92.9%–106.4% and method precision was also good; %CV was less than 8.1%.

1. Introduction

Benign prostatic hyperplasia (BPH) is a noncancerous increase in size of the prostate. Several treatment options exist to treat BPH, including lifestyle changes, medications, self-catheterization, and surgery. Dihydro testosterone (DHT), a metabolite of testosterone, is a critical mediator of prostatic growth. DHT is synthesized in the prostate from circulating testosterone by the action of the enzyme 5α-reductase [1–3]. The three classes of drugs approved for treating BPH include α-blockers, 5α-reductase inhibitors (5-ARIs) and phosphodiesterase 5 (PDE-5) inhibitors. The preferred medical treatment for many men with symptomatic benign prostatic hyperplasia is either an alpha-adrenergic-receptor antagonist (alpha-blocker), which reduces smooth-muscle tone in the prostate, and bladder neck, or a 5α-reductase inhibitor, which reduces prostate volume by inducing epithelial atrophy or PDE-5 inhibitors that promote smooth muscle relaxation and arterial dilation by inhibiting the degradation of cyclic guanosine monophosphate (cGMP) [4–8]. Individually, each class of drug has been studied and proved to improve symptom relief through a variety of mechanisms. Recent focus was on the development of combinational therapies that combine classes of drugs in order to provide maximal benefit.

Over the last decade other combinational therapies have been at the forefront of investigation. One in particular is the combination of tadalafil (TDL), a PDE-5 inhibitor, with finasteride (FNS), a 5-ARI, a safe, effective, and well tolerated treatment for BPH. Evidence suggests that this combination may be particularly effective in reducing treatment-related sexual adverse events associated with 5-ARI treatments [9–11].

Several analytical methods for determination of TDL and FNS in biological matrix were reported. For TDL liquid chromatographic methods (LC) coupled with UV and fluorescence detector [12–14] with lower limit of quantification at 40 ng/ml [13] and at 5 ng/ml [14] require large plasma sample volumes. Gas chromatography/mass spectrometry (GC/MS) methods [15,16] and liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) methods [17–21] were also reported. For FNS, the

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https://doi.org/10.1016/j.jpba.2018.01.020
0731-7085/

Table 1
Method comparison (LC–MS/MS methods).

Method comparison					
Analyte	Biological matrix type,Volume	LLOQ level	Detection, Internal standard, Extraction	Run time	Reference number
TDL	Rat plasma, 100 μl	2 ng/ml	MS, Acebutalol, LLE	4 min	[17]
	Human blood plasma, 50 μl	2 ng/ml	MS, Domperidone,LLE	5 min	[18]
	Human plasma, 20 μl	5 ng/ml	MS, Sildenafil,PPT	1 min	[19]
	Human plasma, 250 μl	10 ng/ml	MS, Sildenafil,LLE	1.2 min	[20]
	Blood, urine 0.5 ml	1 ng/ml	MS,Tadalafil-d3, LLE	15 min	[21]
FNS	Human plasma 200 μl	1 ng/ml	MS, Beclomethasone, LLE	13 min	[27]
	Human serum 200 μl	0.1 ng/ml	MS, Beclomethasone, LLE	10 min	[28]
	Human plasma 100 μl	0.2 ng/ml	MS, Pantoprazole, LLE	4.5 min	[29]
	Human plasma 100 μl	0.1 ng/ml	MS, Tamoxifen, PPT	13.5 min	[30]
TDL,FNS	Human plasma 250 μl	5 ng/ml, 0.2 ng/ml	MS, Glyburide, SPE	2.8 min	Present method

LLOQ-Lower limit of quantification, LLE-Liquid-liquid extraction, PPT-Protein precipitation, SPE-Solid phase extraction.

reported methods include polarography [22], high performance liquid chromatographic methods (HPLC) [23–26] and LC–MS/MS methods [27–30]. Some comparative salient features of reported LC–MS/MS methods for both TDL and FNS are presented in Table 1.

TDL peak plasma concentration (C_{max}) is observed at 2 h after single oral administration. At therapeutic concentration range, 94% of TDL is bound to plasma proteins and metabolized randomly to a catechol metabolite by CYP3A4. This catechol metabolite transforms to methyl catechol and methyl catechol glucuronide conjugate via extensive methylation and glucuronidation, respectively. Methyl catechol glucuronide is the major circulating metabolite. Less than 10% of methyl catechol concentration was observed when compared to methyl catechol glucuronide concentration in circulation. In vitro data suggests that, these metabolites are not expected to be pharmacologically active at concentrations observed. TDL on over doses up to 500 mg, have been given to healthy subjects, and multiple daily doses up to 100 mg have been given to patients but the adverse events were similar to those seen at lower doses. TDL pharmacokinetics are linear with respect to dose and time, the available doses are 2.5, 5, 10, 20 mg. The C_{max} for 20 mg dose of TDL was approximately 352–514 ng/ml [31,32]. The maximum recommended human dose is 40 mg per day. The half-life ($T_{1/2}$) was approximately 17–26 h for single dose and 18.7–40 h for daily (steady state) administration. A steady state of TDL is reached after 5 days of daily administration with a plasma concentration that is roughly 1.6 times higher than that of a single dose [31]. In study simulating pharmacokinetics of TDL, a dosage of 5 mg once in a day was estimated to lead a serum concentration of 55 ng/ml, which corresponds to 90% PDE5 inhibition in vitro [33,34]. The present method is able to quantify the TDL at very low level (i.e. LLOQ 5 ng/ml), which means that the established linear range is suitable to monitor TDL circulating levels across the relevant clinical range up to four terminal half-life's ($T_{1/2}$), right from administration to approximate elimination from the body.

FNS is extensively absorbed from the gastrointestinal tract after oral administration and mean C_{max} concentration is 38.1 ± 7.0 ng/ml for 5 mg dose. The T_{max} is around 1.8 ± 0.8 h. The maximum FNS plasma concentration averaged for 1 mg dose is 9.2 ng/ml. Around 90% of the circulating FNS was bound to plasma proteins. The major metabolites recovered from the urine and plasma were the monocarboxylic acid metabolite and monohydroxylated metabolite respectively. Biotransformation leads to rapid and extensive hepatic oxidative pathways results in formation of crucial inactive compounds eliminated primarily by the bile. The available doses are 1&5 mg. No cases of significant toxicity from isolated FNS ingestion have been reported. FNS is safe and no adverse effects were observed for single doses up to 400 mg and multiple doses up to 80 mg/day for 3 months, 40 mg daily for 24 weeks and fatal dose was not known [35–37]. Slow accumulation of FNS was observed after multiple dosing. After dosing with 5 mg/day of FNS for 17 days, plasma concentrations of FNS was 47% and 54% higher than the first dose in men aged 45–60 years old and ≥70 years old respectively. The mean concentration after 17 days of dosing was 6.2 ng/ml (in between 2.4-9.8 ng/ml) and 8.1 ng/ml (in between 1.8–19.7 ng/ml) respectively for the two age groups. The mean plasma concentration in another study in patients with BPH (mean age, 65 years) receiving 5 mg/day was 9.4 ng/ml (in between 7.1–13.3 ng/ml; n = 22) after a year of dosing. The proposed method is suitable for reproducible quantification of FNS even at sub therapeutic concentration levels.

As of today, no method was reported with full validation results for the simultaneous estimation of TDL and FNS in human plasma. A sensitive and rapid method was developed for estimation of TDL and FNS in single analytical run by using LC–MS/MS and validated fully by CRO (Contract Research Organisation) approach for bio analytical method validation. In the present study the LLOQ achieved is 0.2 ng/ml, 5 ng/ml for FNS and TDL, respectively. The total analytical run time was 2.8 min. This method was suitable for the analysis of plasma samples from clinical trials of TDL and FNS in combination or alone.

2. Experimental

2.1. Chemicals and materials

TDL, FNS and GLB were obtained from Clear Synth Limited, Hyderabad, India. HPLC grade methanol and acetonitrile, analytical grade ammonium formate and reagent-grade orthophosporic acid and *n*-hexane were purchased from Merck Specialities Ltd (Mumbai, India). Water used for the LC–MS/MS analysis was collected from a Milli-Q water purification system procured from Millipore (Bangalore, India). All other chemicals and reagents were of analytical grade and used without further purification.

2.2. HPLC operating conditions

A Shimadzu LC-20 AD Series HPLC system with auto sampler (Shimadzu Corporation, Kyoto, Japan) was used to inject 20 μl sample solution onto a Zorbax eclipse plus C_{18} column (4.6 × 50 mm × 5 μm). The column oven was operated at 35 °C. The mobile phase containing a mixture of 4 mM ammonium formate (pH 4.0) – acetonitrile – methanol 20:45:35 (v/v/v) was filtered through a