Simultaneous determination of ACE inhibitors and dexibuprofen in active pharmaceutical ingredient, formulations and human serum by RP-HPLC

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Abstract: The contemporary work describes a rapid and cost effective reversed phase High Performance Liquid Chromatography (RP-HPLC) method for the quantification of Captopril, Lisinopril and Dexibuprofen (DXP) simultaneously in dosage formulations, active pharmaceutical ingredients and human serum. The chromatographic system included LC-20A pump, Sil-20A auto sampler and SPD-20A UV/visible detector. The estimation was carried out by using a C18 (5µm, 250 ×4.6 mm) column with mobile phase methanol: water (80:20 v/v, pH 3.0) at 230 nm with a flow rate of 1.0 ml·min^{-1}. The retention time of Dexibuprofen was 5.4 min while that of Captopril and Lisinopril were found to be 3.2 and 1.8 minutes respectively. There was no considerable variation exists in between the tested drug spiked in serum and the extent recovered, without interference of serum in concurrent approximation. The method developed was found to be precise, selective and validated for precision, linearity, specificity, accuracy, limit of detection and limit of quantitation. There is no such method reported earlier for the determination of ACE Inhibitors and DXP simultaneously. The present study helps in assessing the co-administration of both drugs in treatment and can be employed for quality control analysis and drug-drug interaction studies.

Keywords: ACE inhibitors, NSAIDs, method validation, RP-HPLC.

INTRODUCTION

Captopril (CAP) and Lisinopril (LSP) belongs to Angiotensin-converting-enzyme inhibitors (ACE inhibitor) that are principally employed for the management of hypertension and congestive heart failure. They causes blood vessels to dilate and decrease blood volume, thereby leading to lowering of blood pressure (Davis et al., 2002). They are also used in diabetic nephropathy and for prophylactic treatment after myocardial infarction. However, duration of antihypertensive action after a single oral dose of CAP is only 6-8 h, so it is recommended to take a daily dose of 37.5-75 mg thrice a day. CAP is most stable at pH 1.2 and it becomes unstable on high pH (Rahman *et al.,* 2006). The suggested dose of LSP is 20-80 mg once daily. It is a lysine analog of enalaprilat, which is the active metabolite of enalapril (Sagirli et al 2004). LSP is not considerably metabolized in humans; hence the drug is largely excreted unaffected in urine. Peak serum concentrations of LSP are reached in about 6 h following administration. Different researchers have reported the analytical methods for estimation of LSP in pharmaceutical dosage forms (El-Gindy*et al.,* 2001; Hillaert *et al.*2001)

Dexibuprofen (DXP) is a non-steroidal anti-inflammatory drug used as symptomatic treatment for osteoarthritis, primary dysmenorrhea, muscular skeletal pain or dental pain. It reduces gastric damage and provide better analgesic and anti-inflammatory effects than racemic ibuprofen (Bonabello *et al.,* 2003). The literature is enriched with a diversity of analytical procedures for determination of DXP in pharmaceutical drugs (Chitlange *et al.,* 2009; Balan *et al.,* 2011)

Numerous studies have been conducted on the interaction between ACE inhibitors and NSAIDs (Polónia *et al.,* 1995; Gurwitz *et al.,* 1996; Sultana *et al*., 2006). Similarly, there are number of possible drug interactions of LSP with NSAIDs (Fogari et al., 2002; Dubey et al., 2005). To the best of authors' knowledge simultaneous estimation of ACE Inhibitors and DXP using HPLC in dosage formulations, active pharmaceutical ingredients and human serum has not been reported earlier. The present study was conducted with the aim to focus on the development and validation of an accurate, rapid as well as a cost effective method for the quantification of ACE Inhibitors and DXP by RP-HPLC method in dosage formulations, active pharmaceutical ingredients and human serum. The developed method was validated as per the ICH (Q2A 1995) guiding principles(Guideline IHT, 1995)

MATERIALS AND METHODS

Materials

Tablet formulations of all active pharmaceutical ingredients (APIs) were purchased from local pharmacy.

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The expiry date of all drugs was evaluated at the time of study. Serum was collected from a healthy person at Fatmid Foundation Karachi. All other reagents used were of HPLC grade.

Equipment

HPLC system (LC 20A, Shimadzu Corp., Japan), Communication Bus Module (CBM 102, Shimadzu Corp., Japan), pump (LC 20A, Shimadzu Corp., Japan), UV-spectrophotometric detector (SPD-20A, Shimadzu Corp., Japan), Class GC software (GC 10, 5.03), Spalco® C18 (5µm, 250 × 4.6mm) and C18 (Purospher ® Star, 5 µm, 250 × 4.6mm). UV-visible spectrophotometer (Shimadzu 1800) was also used for the evaluation of isosbestic and individual point of analytes.

Preparation of reference solution

Ten (10) mg of each API (CAP, LSP, DXP) was used to prepare 100mL stock solutions (100µg/mL) separately. The diluent was used (80:20, methanol: water). Working solutions were further diluted in range of 0.5-50µg/mL for ACE inhibitors and 0.25-25µg/mL for DXP.

Preparation of sample solutions from tablets

For extraction of each active moieties of CAP®, LSP® and DXP® crushed samples of twenty tablets were used separately and then equivalent powder quantity (10 mg) was dissolved in methanol to extract out drugs. Intermittent sonication was performed during 1 hour resting period for each sample. Final solutions were filtered and then serially diluted to prepare working standards in the range of 0.5-50µg/mL for ACE Inhibitors and 0.25-25µg/mL for DXP. Diluted solution were further passed through 0.45 micron filter paper and then injected.

Preparation of serum solutions of drugs

Separation of plasma was performed by shaking and centrifugation at (10,000 rpm for 10min); 1:9 v/v ratio of acetonitrile (ACN) was used for deproteination with repeated centrifugation at same speed (Naveed *et al.,* 2015). Supernatant was collected, filtered, stored (refrigerated) at -20°C and then evaluated for analyte concentration in the range of 0.5-50µg/mL for ACE Inhibitors and 0.25-25µg/mL for DXP.

Chromatographic conditions

The mobile phase used was methanol: water (80:20 v/v) (pH 3). It was filtered before use through 0.45µ membrane filter. The flow rate of pump was set at 1ml/min. The assay was carried out on a C18 column at a temperature maintained at 25ºC. The sample of 20µl was injected and analyzed under isocratic conditions. Chromatograms were recorded at λ_{max} =230 nm using SPD- 10A VP Shimadzu UV-VIS detector.

Method development and validation procedure

Present study was conducted to obtain an innovative, simple and affordable method for the estimation of ACE Inhibitors (CAP and LSP) and DXP. With the intention of selecting a suitable mobile phase for the determination of ACE Inhibitors (CAP and LSP) and DXP isocratic elution was applied. The analytical procedure was optimized by altering the flow rate, composition of mobile phase and pH of the mobile phase. Methanol and water were the mobile phases used in the study. The retention time of DXP was 5.4 min while of CAP and LSP were found to be 3.2 and 1.8 minutes respectively. The optimum resolution was achieved by using mobile phase methanol: water (80:20 v/v) having pH adjusted to 3.0 with phosphoric acid.

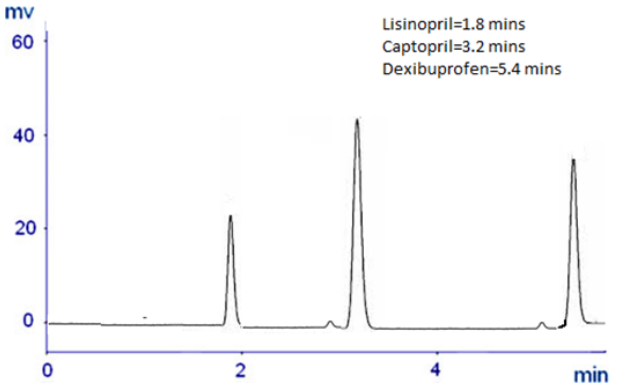


Fig. 1: Chromatogram of ACE Inhibitors (CAP and LSP) and DXP in API

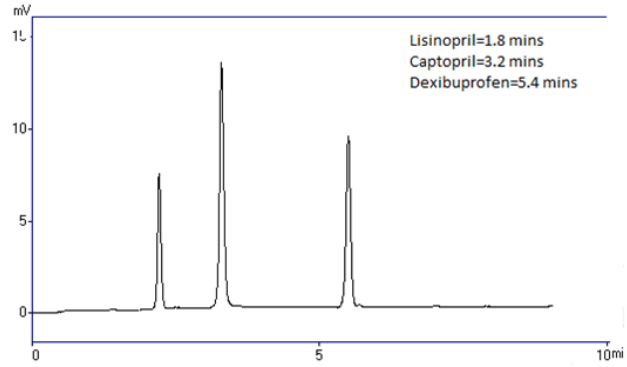


Fig. 2: Chromatogram of ACE Inhibitors (CAP and LSP) and DXP in formulation.

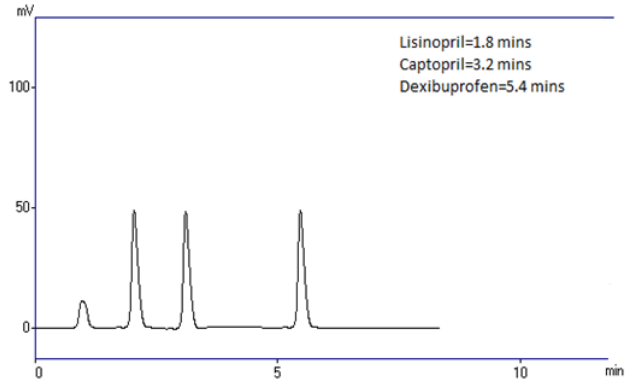


Fig. 3: Chromatogram of ACE Inhibitors (CAP and LSP) and DXP in Serum

Table 1: Chemical structure of ACE Inhibitors (CAP and LSP) and DXP

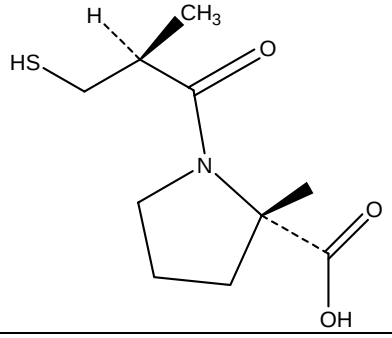
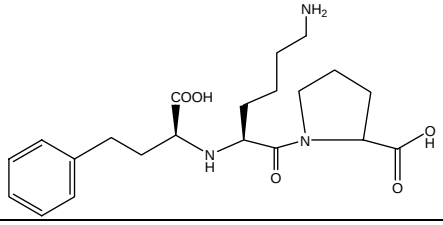
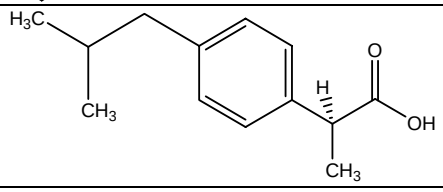
CAP		(2*S*)-1-[(2*S*)-2-methyl-3-sulfanylpropanoyl] pyrrolidine-2-carboxylic acid
LSP		(S)-1-[N2-(1-carboxy-3phenylpropyl)-L-lysyl]-L-prolinedihydrate
DXP		(2*S*)-2-[4-(2-methylpropyl)phenyl]propanoic acid

Table 2: Regression equations with LOD, LOQ.

Drugs	Regression equations	r^2	LOD μgmL^{-1}	LOQ μgmL^{-1}
DXP	y = 1687.x + 21605	0.998	0.003	0.01
CAP	y = 1681.x + 56605	0.981	0.02	0.07
LSP	y = 2018.x + 7146.	0.997	0.001	0.005

LOD = limit of detection, LOQ = limit of quantification, r^2 = correlation coefficient.

Table 3: System suitability of HPLC method for determination of ACE Inhibitors (CAP and LSP and DXP)

Parameter	DXP	CAP	LSP
Retention Time	5.4	3.2	1.8
Height	159433	69686	14680
Resolution	1.889	1.212	1.322
Theoretical Plates (N)	9850.082	10481.79	65500
Capacity Factor (K')	1.454	1.448	1.809

Table 4: Accuracy of ACE Inhibitors (CAP and LSP) and DXP.

Drugs	Conc (μgmL^{-1})	% RSD	Recovery
LSP	25	0.05	24.9
	50	0.06	49.5
	100	0.09	99.8
CAP	25	1.02	24.3
	50	1.03	49.8
	100	10.9	99.9
DXP	25	1.05	24.2
	50	1.06	48.9
	100	0.05	98.9

The HPLC method development and validation was performed according to the official specifications of Centre of Drug Evaluation and Research (CDER-1994), International Conference on Harmonization and United State Pharmacopeias (ICH, 1997, USP, 2008)(Görög, 2008). The method validation parameters included system suitability, linearity, accuracy, specificity, limit of detection, limit of quantification, precision and robustness.

RESULTS

The projected HPLC method involved fewer materials and reagents, simple, less time consuming and may be employed in quality control evaluation in pharmaceutical industries. The chromatograms of ACE Inhibitors (CAP and LSP) and DXP in API, formulations and serum were shown in fig. 1, 2 and 3 respectively.

System suitability and selectivity

The selectivity of the chromatographic method was determined as it is the significant basis for analytical procedures. The method depicted fine resolutions without interference of excipients employed in products. Hence, the method is particular for ACE Inhibitors (CAP and LSP) and DXP (fig. 1, 2, 3).

Linearity

A linear relationship must exist over the range of the analytical procedure. The range of an analytical method lies between the highest and lowest analytical concentrations of a sample.

To evaluate the linearity, limit of detection and limit of quantification (LOQ) of the method, serial dilutions of reference drugs were prepared from the standard stock solution (1000µg/ml) and resolved in a C18 column with UV detector at 230 nm (table 2).

Accuracy and recovery

Percentage recovery of active drug in pharmaceutical dosage form was evaluated for further validation by preparing known amount of drugs at three concentration levels (in triplicate) and injecting to HPLC (table 3, 4).

Precision

The precision interday and intraday reproducibility of the method was established. The intra-and inter-batch precision was estimated by examining the samples (table 5). The values obtained were within the satisfactory range. Hence the method was found to be adequately precise.

Robustness

For evaluating the robustness, the procedure was repeated. Robustness studies performed on method precision, using a sample concentration by creating slight variations in flow rate, change in pH, detection wave length and proportion of methanol.

Quantification limit

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method was evaluated by injecting gradually low concentrations of the standard solutions. The LOD of CAP, LSP and DXP were found to be 0.02, 0.001 and 0.003µg/ml respectively. The LOQ of CAP, LSP and DXP were found to be 0.07, 0.005 and 0.01µg/ml respectively (table 2).

DISCUSSION

We described a widespread, suitable and consistent process for the validation of ACE inhibitors and dexibuprofen in a dosage form. The method has been successfully established and validated for linearity, specificity, accuracy, precision, LOD, LOQ in bulk drug, human serum and pharmaceutical formulation and serum. Results are impeccable and precise and are established by the statistical parameters. There was no interference of excipients or endogenous constituents of serum in analysis, thus no further extraction or separation procedures were required. In summary, the planned method can be used for the drug analysis in routine QC quality control. In addition, this method has the potential application to clinical research of drug combination, multi-drug pharmacokinetics and interactions studies.

CONCLUSIONS

The developed method was innovative, rapid, accurate and can be employed for the determination of ACE Inhibitors (CAP and LSP) and DXP simultaneously for the quality control analysis. Additionally, this method is likely to be applied in clinical research of multi-drug combination, pharmacokinetics and interactions.

REFERENCES

Balan P, Nimila IC, Movva VR, Prasanna ML and Rajasekar SA (2011). Validated Rp-Hplc method for estimation of Dexibuprofen and Paracetamol in combined tablet dosage form. *Int. J. Pharm. Tech. Res*., **3**: 1294-1299.

Bonabello A, Galmozzi M, Canaparo R, Isaia G, Serpe L and Muntoni E et al. (2003). Dexibuprofen (S (+)-isomer ibuprofen) reduces gastric damage and improves analgesic and antiinflammatory effects in rodents. *Anesth. Analg.,* **97**: 402-408.

Chitlange SS, Soni R, Wankhede SB and Kulkarni AA (2009). Spectrophotometric methods for simultaneous estimation of dexibuprofen and paracetamol. AJRC., **2**: 30-33.

CDER- (1994), Reveiwer guidelines: validation of chromatographic methods: Centre of Drug Evaluation and Research.

Davis B, Cutler JA and Gordon D (2002). Major outcomes in high risk hypertensive patients

randomized to angiotensin-converting enzyme inhibitor or calcium channel blocker vs diuretic: The Antihypertensive and Lipid Lowering treatment to prevent Heart Attack Trial (ALLHAT). *JAMA*., **288**(23): 2981-2997.

Dubey K, Balani DK, Tripathi C, Singh R, Bajaj R and Pillai K (2005). Adverse interactions of rofecoxib with lisinopril in spontaneously hypertensive rats. *Clin. Toxicol*., **43**: 361-373.

El-Gindy A, Ashour A, Abdel-Fattah L and Shabana MM . (2001). Spectrophotometric, sepectrofluorimetric and LC determination of lisinopril. *J. Pharm Biomed Anal.,* **25**:913-922

Fogari R, Zoppi A, Carretta R, Veglio F, Salvetti A and Group ICS (2002). Effect of indomethacin on the antihypertensive efficacy of valsartan and lisinopril: A multicentre study. *J. Hypertens.,* **20**: 1007-1014.

Görög S. (2008). Drug safety, drug quality, drug analysis. *J. Pharm. Biomed. Anal.,* **48**: 247-253.

Guideline ICH, (1995). Q2A: Text on Validation of Analytical Procedures: Terms and Definitions. In International Conference on Harmonization, Fed. Reg., (60 FR 11260) (Vol. 1).

ICH (1997). ICH Guideline on Validation of Analytical Procedures: Text and Methodology Q2A, Q2B.ICH.

Gurwitz JH, Everitt DE, Monane M, Glynn RJ, Choodnovskiy I and Beaudet MP *et al* (1996). The impact of ibuprofen on the efficacy of antihypertensive treatment with hydrochlorothiazide in elderly persons. *J. Gerontol. A. Biol. Sci. Med. Sci.,* **51**: M74-M79.

Hillaert S and Van den Bossche W (2001). The quantitative determination of several inhibitors of the angiotensin-converting enzyme by CE. *J. Pharm. Biomed. Anal.,***25**: 775-783.

Polónia J, Boaventura I, Gama G, Camões I, Bernardo F and Andrade P *et al.* (1995). Influence of non steroidal anti-inflammatory drugs on renal function and 24h ambulatory blood pressure-reducing effects of enalapril and nifedipine gastrointestinal therapeutic system in hypertensive patients. *J. Hypertens*., **13**: 925-931.

Rahman Z, Ali M and Khar R (2006). Design and evaluation of bilayer floating tablet of Captopril. *Acta. Pharm*., **56**:49-57.

Sagirli O and Ersoy L (2006). An HPLC method for the determination of lisinopril in human plasma and urine with fluorescence detection. *J. Chromatog. B.,* **809**: 159-165.

Sultana N, Arayne M and Quraishi R (2006). *In vitro* interactions of captopril with NSAID's. *Pak. J. Pharm. Sci.,***19**: 202-207.

Safila N, Farya Z, Huma A, Huma D, Fatima Q, Syeda SA, Nimra W, Safeena N and Zehra A (2015). RP-LC Simultaneous analysis of ranitidine and NSAIDS in active Pharmaceutical ingredient, formulations and human serum. *Lat. Am. J. Pharm.,* **34**(7): 1432-1437.

USP (2008). United States Pharmacopeia. *In*: The United States pharmacopoeial Convention editor. Roukville, USA, The United States pharmacopoeial Convention. pp.1163-1165.