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A Review on: QbD Engineered Method Development and Validation of RP-HPLC for Simultaneous Estimation of Anti-Diabetic Agents.

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Abstract: An isocratic reversed- phase high- performance liquid chromatography(RP- HPLC) system has been developed for rapid-fire and contemporaneous separation and estimation of 3 antidiabetic medicines, videlicet, metformin, pioglitazone, and glimepiride, in mortal tube within 3 min. A reversed- phase RP- HPLC system was developed for the contemporaneous determination of metformin hydrochloride(MET), pioglitazone(PIO), and glimepiride(GLM) in their combined lozenge forms and spiked mortal tube. Quality threat operation principles for determining the critical system parameters (CMPs) and fractional factorial design were made to screen CMPs and latterly, the Box – Behnken design was employed. Literature check reveals good number of logical styles for the estimation of TEN and MET collectively or in combination with other medicines using UV spectrophotometry6- 8, HPLC9- 19, HPTLC20 and LC- MS/ MS21.

The proposed procedure was optimized by espousing a quality- by- design approach. The influence of different factors on chromatographic responses was optimized by applying the two- position full factorial design (25). The optimum chromatographic separation was achieved using Hypersil BDS C18 column at 45 °C, and the mobile phase pumped isocratically composed of methanol potassium dihydrogen phosphate buffer(6.6 mM; pH 7, 6733 v/ v) at an inflow rate of0.814 mL/ min using 235 nm as a discovery wavelength.

Key Words: - RP-HPLC, metformin, pioglitazone, glimepiride, Quality by design, HPLC.

1. INTRODUCTION:

Oral antidiabetic medicines are extensively used for treatment of dia betes mellitus type II. It's the most common diabetes complaint caused by adipose acids and myocells resistance to insulin. Its treatments include insulin secretagogue agents that increase the quantum of insulin buried from the pancreas, insulin sensitizer agents that increase the perceptivity of target organs to insulin, or agents that drop the rate of gastrointestinal tract immersion of glucose. Chemically, metformin (MET) is 1, 1- dimethyl biguanide. It decreases the gluconeogenesis process and increases the glucose uptake by muscles and fat cells. It's the foundation for the treatment of diabetes mellitus type II, where it's used alone or in combination with other antidia betic classes like sulfonylureas, alphaglycosidase impediments, or insulin.

Our literature check vindicated that determination of metfor min has been carried out in tablets by high- performance liquid chromatography(HPLC)(3 – 6), in mortal tube using ion brace HPLC(7), or through using capillary electrophoresis(8, 9). Pioglitazone(PIO) is a thiazolidinedione antidiabetic agent and chemically is(RS)- 5-(4-(2-(5- ethylpyridin-2-yl) ethoxy) benzyl) thiazolidine -2,4-dione. It widely stimulates the peroxisome proliferator- actuated receptor gamma (PPAR- γ) and, to a lower extent, PPAR- α. It's also used for the treatment of diabetes mellitus type II either alone or in combination with other oral anti diabetic medicines.

2. MATERIALS AND METHOD:

MET (99.00), LIN (99.7), PIO (99.5), and GLM (99.7). Excipients included microcrystalline hypromellose, cellulose, magnesium stearate, hydroxypropyl methylcellulose, pregelatinized bounce, lactose monohydrate croscarmellose sodium, pregelatinized bounce, and colloidal silicon dioxide. All the accoutrements used in the trial were gifs from Sigma for medicinal diligence (Moubarak Indus trial Zone, Quesna- Menoufa- Egypt). Mortal tube samples were kindly handed from the blood bank center of Tanta University Hospital afer the needed processes were done. All styles were carried out under applicable guidelines and regulations.

3. CHEMICALS AND REAGENTS:

3.1 HPLC INSTRUMENTAL ATTRIBUTES AND ANALYTICAL SETUP:

The chromatographic analysis of the APIs was performed on the Waters Alliance HPLC system (model no e2695 Waters Co, MA, U.S.A.) having a PDA sensor. The instrument comported of a quaternary detergent director, a bus- sample and a column roaster. Empower software ver.2.0 was used. The analysis was carried out using a reversed- phase C18 column(100-5-C184.6 × 150 mm, 5 μm) from Kromasil Electron Corporation, Nouryon, Sweden. Different wavelengths were used for discovery(278 nm

for CPX and 368 nm for pattern).31 The mobile phase combination of phosphoric acid buffer(pH3.0) and acetonitrile with a rate of 8713 v/ v was eluted at an inflow rate of1.0 mL/ min. The run time for the analysis was 20 min at 40 °C column temperature. Injection volume was taken as 10 µL. A0.45 µm membrane sludge (Millipore) was used for the mobile phase, and a0.22 µm hyp, sludge was used (Avantor performance accoutrements India limited) for chromatographic samples.

3.2 Method Validation:

As per ICH Q2(R1) guidelines, the proposed method was validated with different parameters, system suitability, specificity linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), robustness, as well as stability studies at respective storage conditions.

The stock results of CPX and RUT were independently prepared in a volumetric beaker (10 mL), taking the accurate weight of theirsolid standard to gain 1 mg/ mL attention. The standard stock result of CPX was prepared in a buffersolution of the mobile phase, while pattern was dissolved in methanol and volume acclimated by the mobile phase, followed by sonication (6 min). The working result was prepared by taking 1 mL from the stock result and lacing with the mobile phase to gain 100 µg/ mL of attention. The working result of CPX and RUT was farther serially adulterated to prepare the estimation standard, with attention 1 −15 µg/ mL28 for analytes.

A analogous procedure was followed to prepare quality control samples of 4, 8, and 12 µg/ mL, which were considered as lower quality control(LQC), middle quality control(MQC), and advanced quality control(HQC) results, independently. All results were filtered and lately prepared and stored in the refrigerator.

The method was validated according to the Food and Drug Administration guidelines for bioanalytical methods validation.

3.2.1 HPLC Instrumental Attributes and Analytical Setup:

The chromatographic analysis of the APIs was performed on the Waters Alliance HPLC system (model no e2695 Waters Co, MA, U.S.A.) having a PDA detector. The instrument consisted of a quaternary solvent manager, an auto-sampler and a column oven. Empower software ver.2.0 was used. The analysis was carried out using a reversed-phase C18 column (100-5-C18 4.6 × 150 mm, 5 µm) from Kromasil Electron Corporation, Nouryon, Sweden. Different wavelengths were used for detection (278 nm for CPX and 368 nm for RUT).31 The mobile phase combination of phosphoric acid buffer (pH 3.0) and acetonitrile with a ratio of 87:13% v/v was eluted at a flow rate of 1.0 mL/ min. The run time for the analysis was 20 min at 40 °C column temperature. Injection volume was taken as 10 µL. A 0.45 µm membrane filter (Millipore) was used for the mobile phase, and a 0.22 µm syringe filter was used (Avantor performance materials India limited) for chromatographic samples.

3.2.2 Preparation of Stock Solution, Working Solution, and Quality Control Solution:

The stock solutions of CPX and RUT were separately prepared in a volumetric flask (10 mL), taking the accurate weight of theirsolid standard to obtain 1 mg/ mL concentration. The standard stock solution of CPX was prepared in a buffersolution of the mobile phase, while RUT was dissolved in methanol and volume adjusted by the mobile phase, followed by sonication (6 min). The working solution was prepared by taking 1 mL from the stock solution and diluting with the mobile phase to obtain 100 µg/mL of concentration. The working solution of CPX and RUT was further serially diluted to prepare the calibration standard, with concentration 1−15 µg/mL28 for analytes. A similar procedure was followed to prepare quality control samples of 4, 8, and 12 µg/mL, which were considered as lower quality control (LQC), middle quality control (MQC), and higher quality control (HQC) solutions, respectively. All solutions were filtered and freshly prepared and stored in the refrigerator.

Table 1. Selected Full Factorial Design 3^3 2^2 Variables and Their Constraints

independent variable	levels		
	low (−1)	medium (0)	high (+1)
A = organic phase %(v/v)	7.0	10	13
B = flow rate (mL/min)	0.5	1.0	1.5
C = mobile phase pH	2.9	3.45	4
independent variable	constraint	importance	
A = organic phase %(v/v)	in range	+++	
B = flow rate (mL/min)	in range	+++	
C = mobile phase pH	in range	+++	
dependent variable			
Y_1 = RT of CPX (min)		minimum	+++
Y_2 = number of theoretical plates of CPX		maximum	+++
Y_3 = tailing factor of CPX		minimum	+++
Y_4 = RT of RUT (min)		minimum	+++
Y_5 = number of theoretical plates of RUT		maximum	+++
Y_6 = tailing factor of RUT		minimum	+++
independent variable	constraint	importance	
A = organic phase %(v/v)	in range	+++	
B = flow rate (mL/min)	in range	+++	
C = mobile phase pH	in range	+++	
dependent variable			
Y_1 = RT of CPX (min)		minimum	+++
Y_2 = number of theoretical plates of CPX		maximum	+++
Y_3 = tailing factor of CPX		minimum	+++
Y_4 = RT of RUT (min)		minimum	+++
Y_5 = number of theoretical plates of RUT		maximum	+++
Y_6 = tailing factor of RUT		minimum	+++

3.2.3 System Suitability Test:

System suitability is an integral part of chromatography technique to check method reproducibility and to ensure that the procedure is adequate for the intended use. The test was conducted at MQC concentration (8 µg/mL) to inject six replicas, and the outcome was evaluated by the RT, the peak area, the theoretical column plate. And tailing factors. To ensure the system's suitability, the considerable approved criterion is the relative standard deviation (% RSD) of RT. The range of peak area should be ≤2%. The range of tailing factor should not surpass 2. The range of theoretical plates of the column should not be more than 2000 (N > 2000)

4. ACCURACY AND PRECISION:

Five different attention of the medicine admixture were specified for linearity studies. The estimation angles attained by conniving peak area against attention showed linearity in the attention range of2.50 – 100 µg/ mL for all medicines. Linear retrogression equations for metfor min, pioglitazone, and glimepiride were set up to be y = 61.08 x99.75, y = 12.22 x24.98, and y = 29.62 x41.84, independently, and the retrogression measure values(r) were0.999 for the three medicines, indicating a high degree of linearity.

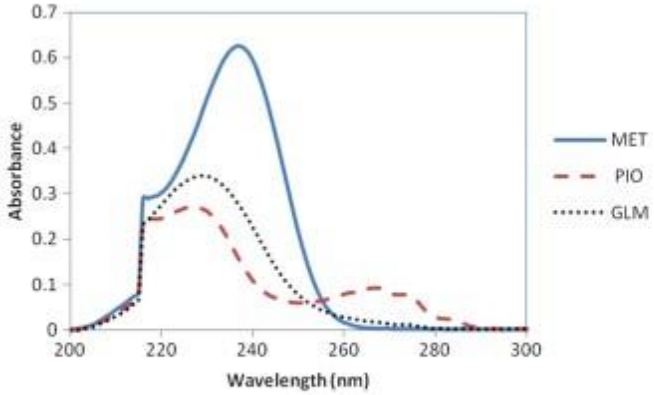

Figure 2. Overlain spectra of 1 µg/mL metformin (MET), pioglitazone (PIO), and glimepiride (GLM) at maximum wavelengths of 237, 227, and 229 nm, respectively

5. SELECTIVITY AND SPECIFICITY:

The selectivity of the system was checked by edging in the results of metfromin, pioglitazone, and glimepiride into the column independently, where 3 sharp peaks were attained at retention time's of1.24, 2.32, and2.77 min, independently, and these peaks weren't attained for the blank result. Also, the particularity studies revealed that the presence of mortal tube did not show any kind of hindrance with the sharp and well- resolved peaks of the three medicines.

6. ROBUSTNESS:

The method's robustness was investigated by checking the impact of minor variations in the experimental parameters on chromatographic responses. The effect of % methanol (67% ± 1%), buffer strength (6.6 mM±0.1), pH (7±0.1), flow rate (0.814±0.1), and temperature (45 °C±1 °C) were examined and showed non-significant effect on % RSD values and % recoveries.

7. APPLICATIONS:

7.1 ANALYSIS OF THE STUDIED DRUGS IN THEIR TABLETS

The proposed system was applied for the determina tion of the studied medicines in their marketable tablets (set Omaril ®, Glucophage ®, and Ezetimibe ®) without hindrance from the common tablet excipients. The attained mean reclamations were set up to be99.86 ±1.01, 100.05 ±1.62, and and99.59 ±1.9 for OMG, MET, and EZT, independently with small values of RSD values attesting the eventuality for effective use of the developed procedure for analysis of the cited medicines in their tablets.

7.2 APPLICATION TO SPIKED HUMAN PLASMA SAMPLES

The studied drugs were simultaneously analyzed in spiked human plasma according to their therapeutic levels. The maximum plasma concentration (Cmax) of OMG was stated to be 0.3 µg/mL within 1 h after an oral dose of 25 mg/day. Maximum MET plasma levels doesn't exceed 5 µg/mL even at maximum doses, while Cmax of EZT was reported to be 0.004 µg/mL after taking a dose of 10 mg daily. The proposed method sensitivity was down to 0.2 and 0.5 µg/mL for OMG and MET, respectively which permitted its successful use for the determination of the two drugs in spiked plasma, while for EZT, this method can be useful for its determination in case of toxicity with over doses.

$P = 1.3941\ C - 0.1884$ ($r = 0.9797$) for OMG

$P = 3.1266\ C - 0.6541$ ($r = 0.9971$) for MET

$P = 0.7625\ C - 0.077$ ($r = 0.9952$) for EZT

Where, P is the peak area, C refers to drug concentration (µg/mL) and r represents the correlation coefficients. Concerning the matrix effect, the proposed approach showed a good selectivity towards the studied drugs in spiked plasma with acceptable values of % recoveries in the range of (94.3-105.7%) for the three drugs and low % RSD values confirming that there was no interference from endogenous plasma components on Rts of the drugs as presented in Additional file.

8. RESULTS AND DISCUSSION:

Assessment of the Analytical Method Using the QbD Approach. In the A QbD approach, the analytical target profile (ATP) plays a similar role as the quality target product profile (QTPP) in specifying the objectives for method development (Table 3). ATP serves as the intended characteristic of the CAA of the method's intended purpose and regulatory constraints. The ATP specifies a collection of attributes and target analytes that must be measured, such as the technique and concentration range that will be employed as per the required characteristics of the method. The analytical method monitoring is associated with critical method parameters (CMPs). CMP and critical quality attributes are connected in a cause-and-effect manner, and it has the ability to influence the selected CAAs. The three most important technical factors affecting the HPLC procedure are the column temperature, the organic phase concentration, and the mobile phase pH. Column aging (CMP), for example, can have an effect on the tailing factor and plate counts (CAA). In this experimental design, tailing factor, theoretical plates, peak area, and RT have been considered as CAAs as shown

9. CONCLUSION:

The presented method was developed and validated for rapid simultaneous estimation of metformin, pioglitazone, and glimepiride within 3 min. The results obtained indicate that the proposed method is rapid, accurate, selective, robust, and reproducible. This analytical method can be also adequate and useful for the clinical estimation of metformin, pioglitazone, and glimepiride in human plasma samples according to the FDA guidelines in respect of pharmacokinetic and bioequivalence studies that would be useful in therapeutic drug monitoring.

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