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RESEARCH ARTICLE

A Green Reverse Phase HPLC Method and it's Validation for Estimation of Chlorthalidone, Olmesartan, and Cilnidipine in Human Plasma: Compatible to LC-MS

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Abstract: A reverse phase HPLC bioanalytical technique was developed and validated to measure the levels of Cilnidipine (CIL), Olmesartan (OLME), and Chlorthalidone (CLT) in human plasma. The HPLC method is a green method that uses a mobile phase made up of 60:40 (%v/v) acetonitrile and 0.1% formic acid as well as a Shiseido cap cell pack C18 Phenomenex 5µ (4.6x250 mm) column as the stationary phase for chromatographic separation. At 220 nm, a PDA detector was employed for quantitation. The flow is 1.0 mL/min. Linearity was noted for all three analytes between 10 and 120 µg/mL. The process was rigorously, precisely, and specifically tested. Next, the simultaneous measurement of cilnidipine (CIL), olmesartan (OLME), and chlorthalidone (CLT) in human plasma is attempted using this environmentally friendly method using LC-MS. To verify the application of LC-MS for the identification of these drugs in biological matrices, the separation was accomplished using a Shimadzu LC-MS-8040 triple Quadrupole apparatus. The identical RP-HPLC with MS detection settings were used for the procedure. Retention durations for Cilnidipine, Olmesartan, and Chlorthalidone were 2.265 min, 3.272 min, and 4.612 min respectively. The devised method was LCMS compatible, and each drug was identified using the same method. The suggested approach can be easily used for regular quality control and pharmacokinetic analysis since it has been validated for linearity, accuracy, precision, LOD, and LOQ as per guidelines.

Keywords: Cilnidipine (CIL), Olmesartan (OLME), Chlorthalidone (CLT), Mass spectrometry, Liquid Chromatography.

INTRODUCTION

Developing green analytical techniques to decrease and get rid of dangerous compounds like organic solvents is a recent trend in analytical chemistry. Choosing a more environmentally friendly solvent system is crucial when developing HPLC methods because they produce a lot of waste organic solvent. The majority of initiatives to make chemical processes greener concentrate on employing safer, less harmful chemicals. It's possible to utilise reagents and auxiliaries less frequently[1]. Utilising a green RP-HPLC technique reduces the amount of organic solvents used. Thus, there is a decrease in the creation of organic waste. Preclinical, biopharmaceutical, and clinical pharmacological research all depend on the effective application of sensitive and selective analytical techniques for the quantitative assessment of pharmaceuticals and their metabolites, or analytes[2]. Green analytical chemistry is an environmental-friendly approach to analytical chemistry that aims to minimize the negative impact of analytical techniques on the environment and human health. A technique utilised for the quantitative determination of the analytes in a given physiological matrix, such as blood, plasma, serum, or urine, is said to be exact and repeatable if it can be demonstrated through validation of a bioanalytical method. LC-MS is a combination of the physicochemical separation capabilities of liquid chromatography (LC) and the mass (m/z) separation and detection capabilities of mass spectrometry (MS or MS/MS). LC-MS is a very sensitive technique, for which assay selectivity can be readily achieved via three stages of separation of the analyte(s) of interest from unwanted components in the biological matrix: (1) sample extraction (protein precipitation, liquid-liquid extraction, solid-phase extraction, etc.), (2) column chromatography and (3) tandem mass spectrometric detection in selected reaction monitoring (SRM) or multiple reaction monitoring (MRM) mode. Chlorthalidone, Olmesartan, and Cilnidipine are prescription medications used to treat hypertension[3].

Chlorthalidone (CLT) is an oral thiazide-like diuretic used to treat hypertension and edoema, including those associated with heart failure. It is also referred to as 2-chloro-5-(1-hydroxy-3-oxoiso-indolin-1-yl) benzene sulphonamide. Chlorthalidone is a thiazide diuretic that increases the production of urine, which helps reduce the body's surplus fluid content[4, 5]. By decreasing the amount of fluid the heart must pump and by widening blood vessels, blood flow is increased, which helps lower blood pressure[6]. Chemical structure is given in Figure: 1 (a).

In terms of chemistry, Olmesartan medoxomil (OLME) is 5-methyl-2-oxo-2H-1,3-dioxol-4-ylmethyl 4-(2-hydroxypropan-2-yl).(2H-1,2,3,4-tetrazol-5-yl) Tetrazol-5-yl-2-5-carboxylate [-phenyl] phenylmethyl-2-propyl-1-1H-imidazole. The hormone known as angiotensin II is prevented from constricting blood

vessels and increasing blood pressure by the angiotensin receptor blocker (ARB) olmesartan[7,8]. Olmesartan inhibits this hormone's action, which helps to reduce blood pressure and relax blood arteries[9]. Chemical structure is given in Figure: 1 (b).

1,4-Dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinecarboxylic acid, or cilnidipine (CIL), is a unique and unusual dihydropyridine calcium channel blocker that is 2-methoxyethyl(2E)-3-phenyl-propenylester[10]. It has a long-lasting, slow-onset vasodilator effect. Blood vascular smooth muscle cells are blocked by calcium channel blockers such as cilnidipine, which helps to relax blood vessels and lower blood pressure[11,12]. Chemical structure is given in Figure: 1 (c).

To better manage blood pressure, these three drugs are frequently taken in combination. A triple therapy for hypertension consists of cilnidipine, olmesartan, and chlorthalidone[13-22]. Sample preparation, LC separation, and mass spectrometry detection are steps in the development of a bioanalytical method for the measurement of chlorthalidone, olmesartan, and cilnidipine utilising the LC-MS technique[23-27]. This method's high sensitivity and specificity allow for the testing of these medications in biological samples. In this study, we reported the development of a simple and rapid method for the detection of chlorthalidone, olmesartan, and cilnidipine using 60 mL of 0.1% formic acid mixed with 40 mL of acetonitrile, as the mobile phase.

Combination therapy is growing enormously to decrease the number of medications for a single disease or their associated diseases. In clinical research, estimation of concomitant drugs plays a key role to study the drug-drug interactions. The research in the current article has undertaken to provide an accurate method which can be applied to estimate chlorthalidone, olmesartan, and cilnidipine which are prescribed as combination therapy. Triple quadrupole mass spectrometry (LC-MS) analysis was carried out after the extraction procedure. This method has a low detection limit, a wide detection range, and is sensitive and accurate. Although there are numerous techniques for determining the medicines understudy alone or in combination[28-30], a literature review showed that there was not a single technique for determining all three drugs simultaneously in human plasma using HPLC. Consequently, an HPLC method was created to measure chlorthalidone, olmesartan, and cilnidipine in human plasma. The method was also created to be compatible with LC-MS, making it simple to conduct LC-MS research in the future. Literature survey revealed some existing LC methods which are stability indicating and applied to fixed dose formulations at high concentrations[31]. Therefore the focus was on developing a green method with high sensitivity that is applicable to plasma samples. Fixed-dose combinations, containing two or more active pharmaceutical substances within a single product, offer advantageous for patients ensuring safe and effective treatment with precise drug combinations. Moreover, LC-MS compatible methods are advantageous for such fixed-dose combinations[32,33].

EXPERIMENTAL:

Reagents and Chemicals:

The source of the blank human plasma sample was Loba Chemicals in Mumbai, India. Gift samples of chlorthalidone, olmesartan, and cilnidipine were acquired from Madras Pharmaceuticals in Chennai, India. The source of standard samples was Madras Pharmaceuticals in Chennai, India. Acetonitrile, methanol, formic acid, and zinc sulphate aqueous solution were purchased from Loba Chemicals in Mumbai, India.

METHOD OPTIMIZATION:

Preparation of Calibration standards and quality control samples:

Blood was extracted, placed in heparinized glass tubes, and centrifuged at 6000 rpm. Plasma that was separated was kept at -20°C.

The three analytes for quantification were chlorthalidone, olmesartan, and cilnidipine. The standard stock was made in methanol and maintained at 2–8°C (1 mg/mL). Next, appropriate aliquots of the stock solution were added to drug-free plasma, resulting in plasma calibration standards with a final concentration of 10 to 120 µg/mL for chlorthalidone, olmesartan, and cilnidipine. To prepare quality control samples, the appropriate aliquots of the drug solutions to drug-free plasma were employed. The QC samples that were aliquoted were the lower quality control (LQC), medium quality control (MQC), and higher quality control (HQC) samples.

A 100 µL plasma sample was aliquoted into a micro centrifuge tube and spiked with 150 µL of methanol and 50 µL of an aqueous solution containing 10% zinc sulphate. Following a one-minute vortex, the samples were kept at 4°C for fifteen minutes. centrifuged for 10 minutes at 14,000 rpm after that. A solution of 20 µL was injected.

40 mL of acetonitrile and 60 mL of 0.1% formic acid were combined, then the mixture was filtered using a 0.22µ membrane filter.

Instrumentation and Chromatographic conditions:

The Shiseido cap cell pack C18 Phenomenex 5µ, (4.6 x 250 mm) column, SPD-M20 PDA detector, electrospray ionisation (ESI) interface, and SIL-20AC autosampler were used in the HPLC-MS/MS Shimadzu LC-MS-8040 triple quadrupole system analysis. The temperature of the column was set to 40°C. The procedure involved setting the flow rate to 1.0 mL/min at sample temperature of 4°C , injecting 20 µL, and using 0.1% formic acid combined with 40 mL of acetonitrile (@60:40) as the mobile phase.

The analytes were separated at 40°C on a Shiseido cap cell pack C18 Phenomenex 5µ (4.6 x 250 mm) stationary phase. The HPLC-MS/MS was operated in two modes (positive and negative ions) via the ESI interface. The analytes were measured using the multiple reaction monitoring (MRM) method. The positive ionisation approach was used to configure the mass spectrometer. The desolvation and block temperatures were maintained at 250 and 400 °C, respectively, with a 4.5 kV detector voltage and 230 kPa of CID gas. Nitrogen was employed as the carrier gas (2.7 L/min) and drying gas (12 L/min). Specific transitions were monitored for Cilnidipine (m/z 492.52 to 318.95), Olmesartan (m/z 558.58 to 349.00) and Chlorthalidone (m/z 338.766) in the quantitative analysis.

Method validation

The method was assessed based on the USFDA criteria for the validation of bio-analytical techniques for selectivity, precision, accuracy, linearity, sensitivity, matrix effect, and stability. The qualities of an analytical method that allow it to identify and measure the analyte in the absence of other components in the sample are called selectivity and specificity. Blank plasma samples from six different donors were examined in order to assess endogenous interference at the retention time at lower quantitation limit (LLOQ).

A comparison of spiked quality control samples (LQC, MQC, and HQC) is necessary to determine whether matrix components are responsible for ion enhancement or suppression, which could lead to inaccurate results, since blood-derived products might contain endogenous chemicals.

An average of six measurements at eight distinct concentration levels—between 10 and 120 µg/mL for chlorthalidone, olmesartan, and cilnidipine was used to assess the linearity of each drug. Six replicates of samples of three concentration levels (20, 60, and 100 µg/mL for chlorthalidone and Olmesartan, for Cilnidipine, respectively) were prepared as low (LQC), medium (MQC), and high (HQC) quality controls. The precision was expressed as a percentage of the relative standard deviation (%RSD) of the regressed concentration. Intra- and inter-day precision experiments were used to evaluate the precision of the approach. Regression concentration, expressed as a % of nominal concentration, was used to assess the method's accuracy. The calibration curves were constructed by plotting each respective peak areas against the concentrations of each analyte, using the weighting factor of $1/x^2$.

Stability studies:

The analyses that followed evaluated the stability of cilnidipine, olmesartan, and chlorthalidone in biological matrix and working solutions under varied storage conditions; the results were presented as a percentage of bias. In order to study the freeze-thaw (3 cycles), bench-top stability, long-term (-20°C), short-term (room temperature), and stability samples (room temperature), six replicates of QC samples were injected into the same matrix of stability samples. Within +15% of the nominal concentration, the stability samples utilised for QC testing yielded results.

Stock solution stability:

The analyte and internal standard stock solution stability were evaluated at the LLOQ and ULOQ levels. Analytes were weighed and put in fresh stock solutions. The corresponding LLOQ and ULOQ stock dilutions for the analytes were then prepared. Six replicates of both fresh and stable samples were fed into the system, along with the stock dilutions from the stocks that were kept at 2–8°C. The stock dilutions were removed and diluted to LLOQ and ULOQ level concentration.

Long-term stability:

The analyte stock solutions were prepared independently and kept between 2 and 8 degrees Celsius. These solutions were further diluted and stored at room temperature in order to acquire analytes at LQC and HQC. Six injections were given in duplicate for the LQC and HQC samples.

Bench top stability:

Each analyte was kept in a deep freezer in six aliquots. After eight hours, the samples were taken out of the freezer, placed on a bench-top, and analysed against a set of recently made calibration curve samples.

Freeze-thaw stability:

Four freeze-thaw cycles were finished by comparing the stabilities with the newly made samples. The samples were kept in the freezer for a minimum of 12 hours in between each freeze-thaw cycle.

Wet extract stability:

After taking stability samples out of the freezer, they were processed using the suggested sample extraction method, put into auto-sampler vials, and allowed to stand at room temperature for 36 hours before being put back into the auto-sampler and checked against newly made calibration standards.

Sensitivity

The sensitivity of the approach was evaluated using the detection limit (signal-to-noise ratio of 3:1) and the limit of quantitation (signal-to-noise ratio of 10:1).

RESULTS:

Method development:

To identify CIL, OLME, and CLT in human plasma, the primary objective of the current work was to develop and fully validate an LC-MS/MS approach. A range of columns and mobile phases with varying pH and strengths were utilised to assess the selectivity and sensitivity in a short run time in order to give the proper chromatographic conditions.

Table I: Regression characteristics for CLT, OMLE and CIL

Parameters	**Chlorthalidone**	**Olmesartan**	**Cilnidipine**
Linearity range (µg/mL)	10-120	10-120	10-120
Detection limit (µg/mL)	1.3046	1.3977	3.1481
Quantitation limit (µg/mL)	10.0655	9.9772	10.1377
Correlation coefficient (r)	0.9991	0.999	0.9995

Table II: Precision results of the proposed method

Chlorthalidone				
QC Levels	**Added concentration (µg/mL)**	**Conc. found (mean ± S.D.)**	**Intra-day Recovery (%) ± RSD (%)**	**Inter-day Recovery (%) ± RSD (%)**
LQC	20	20.775± 0.264	94.0 ± 3.835	90.0 ± 4.175
MQC	60	60.283 ± 0.611	96.1 ± 3.091	92.0 ± 4.947
HQC	100	100.435 ± 0.742	99.2 ± 0.775	93.8 ± 4.789

Olmesartan				
QC Levels	**Added concentration (µg/mL)**	**Conc. found (mean ± S.D.)**	**Intra-day Recovery (%) ± R.S.D. (%)**	**Inter-day Recovery (%) ± R.S.D. (%)**
LQC	20	20.19± 0.335	92.1 ± 2.167	91.1 ± 2.571
MQC	60	59.78 ± 0.560	95.2 ± 2.791	92.1 ± 4.749
HQC	100	100.30± 0.840	99.2 ± 1.875	92.8 ± 4.987

Cilnidipine				
QC Levels	**Added concentration (µg/mL)**	**Conc. found (mean ± S.D.)**	**Intra-day Recovery (%) ± R.S.D. (%)**	**Inter-day Recovery (%) ± R.S.D. (%)**
LQC	20	20.18± 0.201	93.3 ± 2.764	92.9 ± 2.658
MQC	60	59.66 ± 0.480	96.6 ± 3.198	94.2 ± 2.236
HQC	100	99.723 ± 0.553	96.3 ± 2.576	98.1 ± 4.787

Table III: Summary of stability testing of Chlorthalidone, Olmesartan, and Cilnidipine in human plasma under various conditions

Parameter	**QC levels**								
	Chlorthalidone			**Olmesartan**			**Cilnidipine**		
	LQC	**MQC**	**HQC**	**LQC**	**MQC**	**HQC**	**LQC**	**MQC**	**HQC**
(a) Freeze thaw cycles									
Mean	21.188	61.386	101.3	21.87	59.70	101.14	20.161	59.708	100.80
S.D	0.3213	0.685	1.066	0.232	0.545	1.415	0.2446	0.545	1.372
R.S.D	1.5165	1.115	1.053	1.062	0.913	1.399	1.2134	0.913	1.3617
(b) Benchtop stability									
Mean	20.026	60.97	101.01	20.34	60.78	100.08	21.02	60.95	100.79

S.D	0.1084	0.9018	0.7635	0.3612	0.930	1.321	0.337	0.806	1.162
R.S.D	0.5412	1.4791	0.7559	1.7758	1.5302	1.3201	1.606	1.322	1.1533
(c) Short term stability (room temp.)									
Mean	20.548	60.971	101.01	20.648	60.788	100.08	20.548	60.95	100.79
S.D	0.3768	0.9018	0.7635	0.3879	0.9302	1.321	0.3747	0.8062	1.162
R.S.D	1.8337	1.4791	0.7559	1.879	1.5303	1.320	1.823	1.3225	1.153
(d) Long-term(-20℃/2w)									
Mean	20.805	50.492	101.15	20.77	60.74	100.44	20.73	60.77	100.36
S.D	0.2609	0.3180	0.9291	0.26	0.17	1.170	0.300	0.268	0.6346
R.S.D	1.2544	0.5258	0.9184	1.27	0.29	1.165	1.447	0.442	0.6323

SD: standard deviation, n=3, LQC: low-quality control, MQC: Middle-quality control, HQC: High-quality control, RSD: Relative standard deviation.

FIGURES

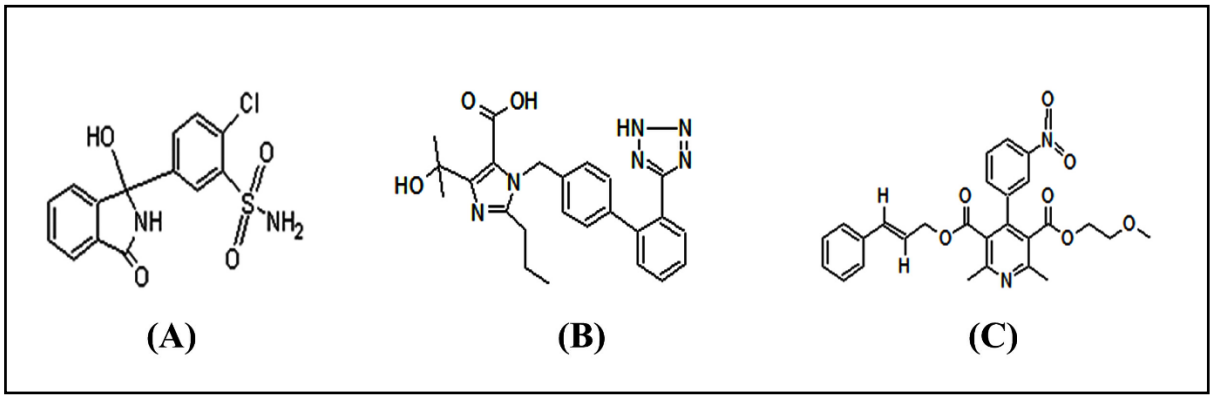

Fig. 1 Chemical structures of (A) Chlorthalidone, (B) Olmesartan, (C) Cilnidipine

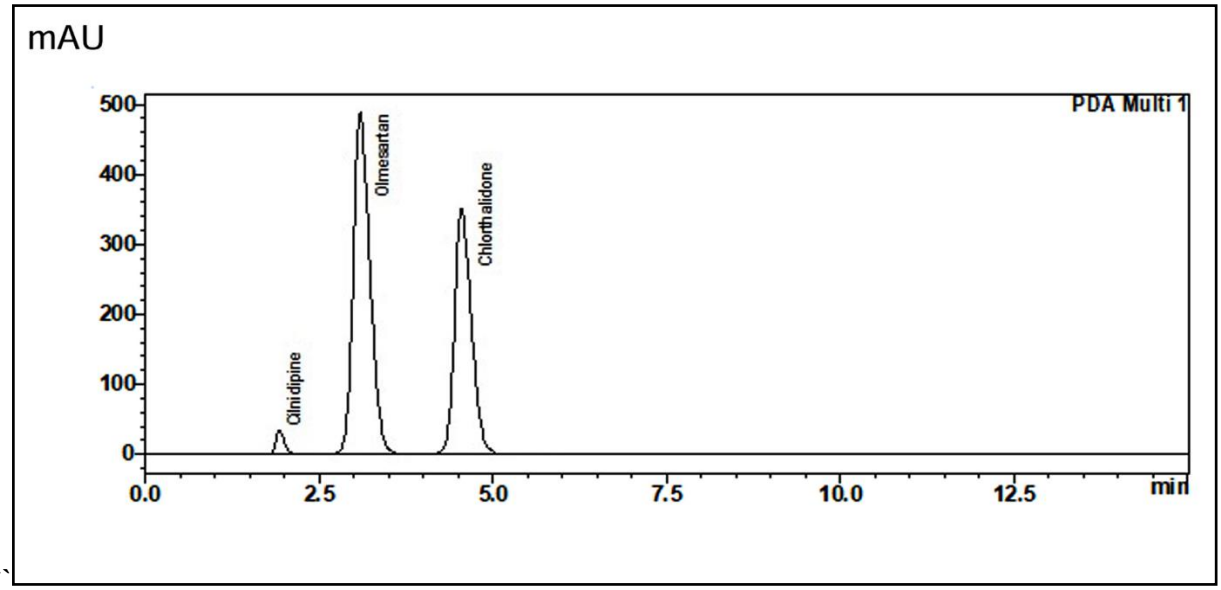

Fig. 2: HPLC chromatogram of Cilnidipine, Olmesartan, and Chlorthalidone

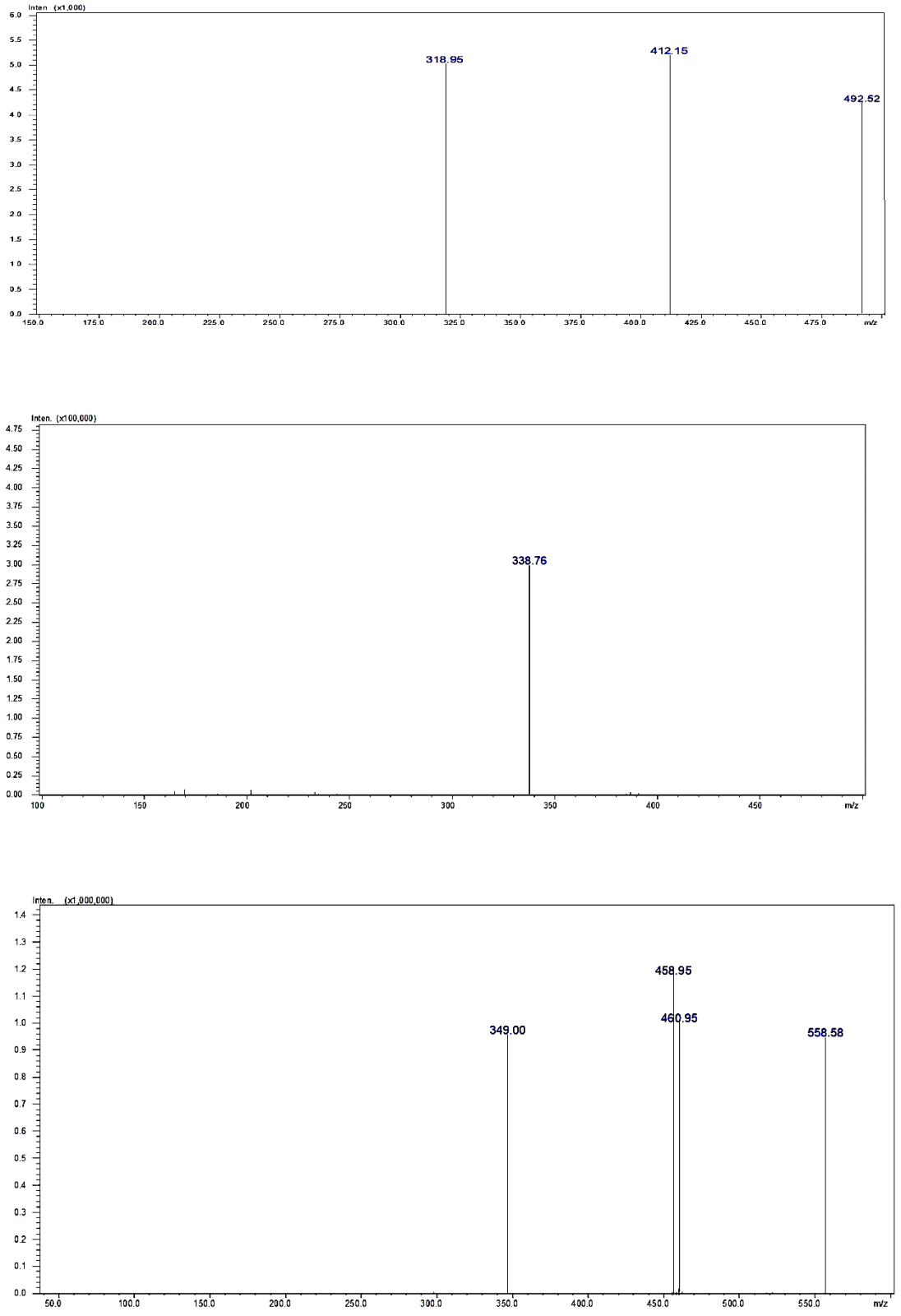

Figure: 3 Standard LC-MS spectra of (A) CLT (B) OMLE (C) and CIL

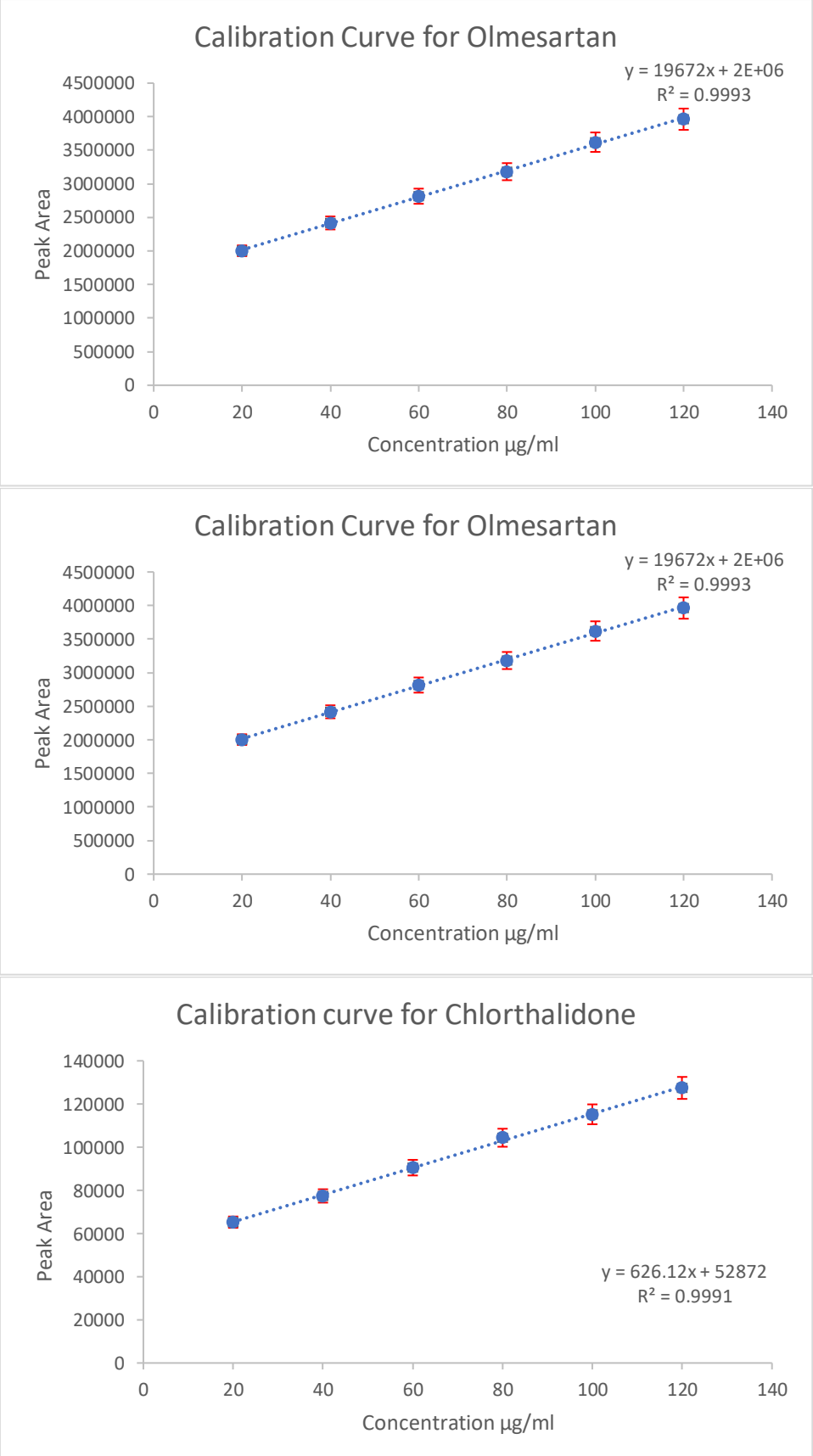

Figure. 4: Calibration curve for Cilnidipine, Olmesartan and Chlorthalidone

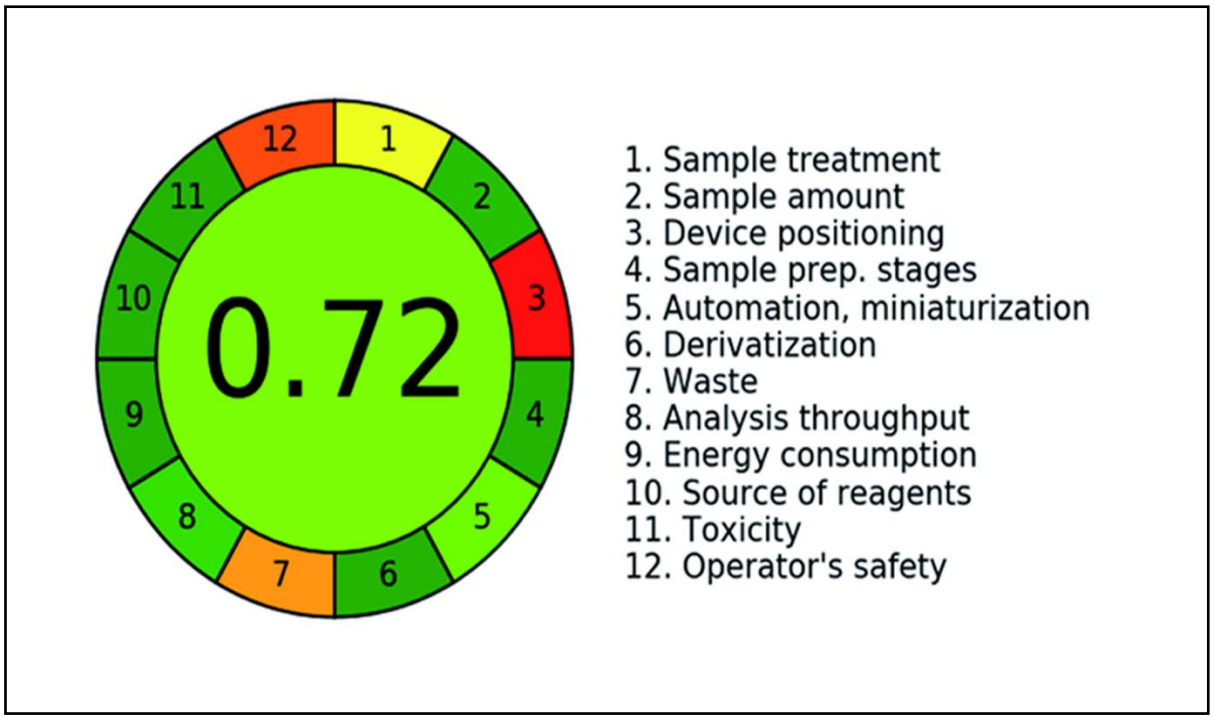

Figure 5: Pictogram of AGREE greenness calculator for Proposed HPLC method

Research has been done on both short- and long-term stability. The results were analysed and recorded. The mobile phase, flow rate, column type, and injection volume were among the chromatographic parameters that were optimised. Different ratios of mobile phases were tested.

Ultimately, the procedure was executed by modifying the flow rate to 1.0 mL/min at 40°C, injecting 20 µL, and using 0.1% formic acid combined with 40 mL of acetonitrile (60:40) as the mobile phase. The measured interface voltage was 4.5 kV. Cilnidipine, Olmesartan, and Chlorthalidone had retention times of 2.265, 3.272, and 4.612 minutes, respectively indicating excellent separation efficacy. Additionally, there were no endogenous peaks interfered with the chromatogram indicating high selectivity and specificity ensuring precise quantification with no interference from biological matrix components.

The chromatograms were given in Figure: 2

Mass spectrometric detection:

The chosen medications were dissolved in methanol and then injected into the LC-MS to enhance the ionisation modes for CLT, OMLE, and CIL. Using the ESI method, the ions of the analytes CLT, OMLE, and CIL were created. While CLT, OLME, and CIL produced $[M+H]^+$-ions in the positive ionisation mode, respectively, a great effort was made to quantify all three molecules in this mode, and the same was applied for the simultaneous estimation of all three analytes. CLT m/z 492.52 →318.95, OMLE m/z 558.58→458.95, and CIL m/z 338.76 were the mass transitions used for quantification (Figure 3).

Method validation

Specificity

By injecting 100 µg/mL concentration solutions of the sample solution, working standards, blank, and placebo separately, the specificity of the devised analytical method was assessed in order to identify any interference from the representative peaks.

Linearity

The linearity of the approach was evaluated for chlorthalidone, olmesartan, and cilnidipine using triplicate measurements at different concentration levels ranging from 10 to 120 µg/mL. Table 1 showed a straight calibration curve with an intercept value and a regression coefficient (> 0.99). Cilnidipine, Olmesartan, and Chlorthalidone were found to have r^2 values of 0.9997, 0.9993, and 0.9991, in that order.

The calibration graph was given in Figure: 4

System suitability:

During the validation process, accuracy and precision were assessed for each of the three analytes (CLT, OMLE, and CIL) using lower, intermediate, and higher quality controls. The values to represent the area response, 'a' the intercept, 'b' the slope, and 'c' the concentration. The mean, n=3, standard deviation (SD), and regression equation (A = a + bc) of the data in Table 1 are shown.

Accuracy and precision

The approach's accuracy and precision were evaluated by measuring the intra-day and inter-day precision at three distinct QC levels using six repetitions. The percentage nominal concentrations ranging from 90.0% to 93.8% (inter-day) and 94.0% to 99.2% (intra-day) were used to establish the mean accuracy level for cilnidipine, olmesartan, and chlorthalidone. It was found that the average precision level was within the allowable range of 15%. The results demonstrate the accuracy and dependability of the methodology developed for the investigation. A summary of the accuracy and intra- and inter-day precision data is given in Table 2.
The data below are presented as mean, n=3, standard deviation (SD), and relative deviation from the mean (RSD).

Stability

It is necessary to confirm the drug's integrity while it is being kept and examined. Three QC values were determined for each of the freeze-thaw, short-term, long-term, and stability samples at six replicates. The results showed that handling the human samples containing CLT, OMLE, and CIL under typical laboratory conditions could be done without experiencing any significant stability issues. Table 3 provided an explanation of the findings.

Sensitivity

The lowest limit detected at 3 µg/mL was found to be the limit of detection for this method based on the signal-to-noise ratio of 3:1. Because of the higher sensitivity of the approach, the quantification was performed at 10 µg/mL. At low doses, this method was found to have an acceptable recovery rate.

Robustness

The robustness of the proposed HPLC approach was demonstrated when intentional changes in the organic strength (2.0%) of the mobile phase did not significantly affect the peak's retention period (0.03 min).

System suitability studies

Peak asymmetry, resolution, and total number of theoretical plates were found for the working solutions. The technique was found to be appropriate for assessing CLT, OLME, and CIL in human plasma, according to the results.

DISCUSSION

To build an effective and workable LC-MS approach, several buffer ratios at different flow rates were utilised to evaluate Cilnidipine, Olmesartan, and Chlorthalidone in human plasma. There aren't many HPLC methods available for estimating the amounts of Cilnidipine, Olmesartan, and Chlorthalidone in formulations utilising RP-HPLC methods; however, there isn't one available for these medications together. The available technology showed that it could detect and measure chlorthalidone, olmesartan, and cilnidipine in human plasma at lower quantities while maintaining sensitivity. The current method is proved for its ruggedness with sufficient recovery and low matrix effect in a simple protein precipitation method with methanol. This method can be applied in studying the preclinical as well as clinical sample analysis of these 3 drugs given to the patients. The linearity range was 10 to 120 µg/mL, with regression coefficients of 0.9997, 0.9993, and 0.9991 for cilnidipine, olmesartan, and chlorthalidone, respectively. The results demonstrated that the technique is linear for the simultaneous estimation of all three medicines, with an R^2 value of 0.999 for each drug. A recovery rate of more than 90% at the LQC, MQC, and HQC levels suggests that the extraction process used is as efficient as possible and repeatable. The quality control values are within the limit for three analytes, as Table 3 illustrates.

Thus, the three analytes were distinctly resolved from one another by the LC method followed with high selectivity. Further the absence of interfering peaks from endogenous compounds indicates the method's specificity demonstrating that the method was well optimized and reproducible. Selectivity of the method in human plasma was evaluated in twelve individual matrix lots along with one lipemic and one hemolytic lot. Peak responses in blank lots were compared against the response of spiked LLOQ and negligible interference was observed at the retention time of analytes. Matrix effect is a special phenomenon associated with LC–MS determination of drugs from biological fluids such as plasma and other matrices. Endogenous components extracted from plasma may suppress or enhance ionization of the analytes in electrospray source if they co-elute with the analytes. It is for this reason that matrix effect was evaluated under the experimental conditions used in this study. The average ion suppression or enhancement at low QC and high QC levels was < 8%, suggesting that matrix effect on the analysis was negligible.

Greenness Evaluation of HPLC method Using AGREE

It is an open-source and downloadable from https://mostwiedzy.pl/AGREE. With numbers 1 through

12 positioned along the edge, the AGREE software generates a clockwise circular figure that represents the 12 tenets of green analytical chemistry. The outcomes of the 12 principles are based on an overall 0–1 scale that is determined by the inputs and weights that are supplied[34, 35]. The total of all 12 principles is the score. The suggested method's AGREE diagram is displayed in Figure 5. The proposed HPLC's representative AGREE score was determined to be 0.72, indicating a significantly higher level of greenness compared to previously published methods.Hence our method demonstrates reduced waste generation and optimized energy consumption in contrast to the methods used without integrating green chemistry principles[36]

CONCLUSION

The resulting strategy proved to be more straightforward, less expensive, and easy to understand than the tedious previously reported approaches. It also made use of the protein- precipitation process. Because of the simple analyte quantification process and rapid, one-step plasma preparation, the method is cost-effective and suitable for the analysis of several samples. Because of its increased sensitivity, the novel method can be applied to clinical and preclinical pharmacokinetic studies that track drug levels in the plasma. It was discovered that the validation protocol's acceptance limit for every parameter and the results were met. After the method was fully validated, all of the method validation parameters showed data that was adequate.

The technique was discovered to be precise. The technique appears to be exact based on the low %RSD readings for method precision. Over a broad concentration range, the linear response for the analyte peak's linearity evaluation was good. Because LCMS compatible buffers are used, the method can be used for the simultaneous determination of Chlorthalidone, Olmesartan, and Cilnidipine from pre-clinical and clinical samples in both HPLC-MS and HPLC-PDA systems. The method's linearity, precision, and accuracy demonstrate that it is specific, accurate, and easily reproducible. Based on the results, we can conclude that the present method is suitable for quantification of multiple analytes simultaneously without any interference and matrix effects. The concomitant drug analysis along with the target analyte is more advantageous with significant environmental sustainability and optimised energy consumption than single compound analysis and also useful in drug interaction and toxicology studies.

AUTHORS CONTRIBUTIONS

All the authors are equally contributed throughout the research.

CONFLICTS OF INTERESTS

All authors declare no conflict of interest.

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