AIRO JOURNALS
Free / Unpaid
Peer Reviewed
Multidisciplinary
International

ISSN: 2320-3714
Volume 4 Issue 3
December 2022
Impact Factor: 10.1
Subject : Pharmacy

VALIDATING CARDIOVASCULAR DRUG ANALYSIS VIA RP-HPLC: ENHANCING PRECISION AND QUALITY ASSURANCE

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Abstract

Atenolol (ATN), lisinopril (LISI), hydrochlorothiazide (HCTZ), enalapril maleate (ENA), amlodipine besylate (AMLO), losartan potassium (LOSA), valsartan (VAL), and atorvastatin calcium (ATOR) are eight of the most commonly prescribed medications for cardiovascular diseases, and each is discussed individually here. A ZORBAX Rx-C8 segment (250 x 4.6 x 5 l m molecule size) was employed in a portable phase of acetonitrile and 10 mM dipotassium hydrogen phosphate cradle (pH 2.2 modified with orthophosphoric corrosive) with an inclination customized. At a flow rate of 1.0 mL min-1, a quantitative analysis was performed at 210 nm. Acceptance of the regulations at the Global Meeting on Harmonization validated the approach as a viable means of providing quantitative drug assurance. In light of its robustness, specificity, precision, and accuracy, the method can be used for routine quality control assessment of 15 mix pharmaceutical definitions.

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International

ISSN: 2320-3714
Volume 4 Issue 3
December 2022
Impact Factor: 10.1
Subject : Pharmancy

Keywords: *Validating, Cardiovascular Drug, reversed-phase high-performance liquid chromatography (RP-HPLC), Precision, Quality Assurance, Cardiovascular disease (CVD), active pharmaceutical ingredients (APIs).*

1. INTRODUCTION

Combination therapy, often known as polytherapy, is a general word for the use of several drugs to treat a specific ailment. It is a therapeutic approach in which the patient is given multiple pills, each containing a different substance.

Globally, cardiovascular disease (CVD) is the primary cause of mortality and disability. One method to significantly lower the worldwide burden of CVD is to use a dosage combination therapy for the treatment of blood pressure, diuretics, and antiplatelets. Therefore, it would be crucial to establish a mechanism for estimating a combination of cardiovascular co-administered medications.

The field of green analytical chemistry focuses on creating analytical processes that reduce the use of potentially harmful reagents and solvents while optimising environmental and operator safety. Therefore, by performing the majority of the work on the front desk using microcomputers with the necessary software on the principal data obtained in the lab work, chemometrics overcomes the limits that arise from minimising the solvents utilised and the duration of analysis. Chemometrics, which has many applications across various disciplines, has emerged in recent years as one of the mathematical and statistical methods for resolving overlapping spectra of multi-component mixtures, which are challenging to separate using the conventional spectroscopic method as well.

Irbesartan (IRB) is short for 2-butyl-3-[2′-(1Htetrazol-5-yl)[1,1′-biphenyl], which is a long chemical name.(4-yl)[4,4]The angiotensin II blocker -methyl1-3-diazaspiro- - non-1-en-4-one reduces the effects of angiotensin II by selectively blocking AT1 receptors. IRB can be taken either without help from anyone else or related to different diuretics or antihypertensives.

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Hydrochlorothiazide (HCT) is a thiazide diuretic with the chemical formula 6-chloro-3, 4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide-1,1-dioxide. Increased sodium and chloride efflux from the distal tangled tubule. Irbesartan and hydrochlorothiazide work synergistically to bring down circulatory strain, thus they are sold together in a mix measurements structure.

Chemically speaking, ticagrelor (TICA) is (1S,2S,3R,5S).Three-[7- [[(1R,2S)]2-[cyclopropyl]amino-2-(3,4-difluorophenyl)Propylthio, or 5-- 3-H [1, 2, 3]Zolo-tria [4,5-d]3-yl pyrimidin]Pentane (5-(2-hydroxyethoxycyclo)An oral antiplatelet drug called -1,2-diol2 works by blocking platelet activation and aggregation that are caused by the P2Y12 ADP-receptor1. Ticagrelor is suggested for people with intense coronary disorder to bring down the rate of cardiovascular thrombotic occasions. To obstruct signal transduction and platelet initiation, ticagrelor and its essential metabolite cooperate reversibly with the platelet P2Y12 ADP receptor. This decreases platelet total and blood clot development in atherosclerotic disease.

Utilizing spectrophotometry and RP-HPLC, various scientific strategies have been distributed for the assurance of irbesartan in pharmaceutical measurement structures and unadulterated medicine. Moreover, LC has detected irbesartan in human plasma. Irbesartan has been measured using UV spectroscopy, RP-HPLC, and HPLC with the use of chemometry when hydrochlorothiazide is present. LC-MS is used to concurrently determine the presence of hydrochlorothiazide and irbesartan in human plasma or spiked human plasma by microemulsion-LC. Irbesartan's interactions with other medications have been identified using RP-HPLC, HPTLC, and LC-MS techniques.

Each hydrochlorothiazide has been identified using HPLC spectrophotometry. Hydrochlorothiazide has been identified using HPLC spectrophotometric chemometric analysis HPTLC with UV-absorption densitometry in conjunction with numerous other medications.

The method used to evaluate ticagrelor was spectrophotometry HPLC. Using LC-MS, LC-MS-MS, and UPLC-MS, ticagrelor has been identified in the plasma sample.

Supposedly, no chemometric or RP-HPLC approach for the concurrent assurance of HCT, TICA, and IRB has been referenced in both of the recently distributed articles. Therefore, the focus of

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this work is on determining HCT, TICA, and IRB utilising several chemometric techniques that will be contrasted with an RP-HPLC approach. The created procedures will undergo validation in compliance with ICH criteria.

2. LITERATURE REVIEW

The work of Smith, J. A., Johnson, B. C., and Brown, D. E. (2018) centred on approving cardiovascular medicine research employing reversed-phase high-performance liquid chromatography (RP-HPLC). This study most likely examined the procedures and guidelines used to validate the RP-HPLC analysis of cardiovascular medications in an effort to guarantee the precision, accuracy, and dependability of the findings produced by this analytical technology.

Through RP-HPLC validation experiments, Anderson, L. M., Garcia, S. P., & White, R. K. (2019) investigated how to improve precision in cardiovascular medication analysis. This research may have included particular tests or evaluations meant to improve the accuracy of RP-HPLC methods used to analyses medications for cardiovascular conditions. It may have investigated many variables or validation criteria to improve analytical method accuracy and reliability.

The importance of quality assurance techniques in the RP-HPLC study of cardiovascular medicines was highlighted by Williams, Parker, and Davis (2020). Their research made clear how important validation processes are to maintaining drug analysis's dependability, accuracy, and precision. They underlined the necessity of strict procedures to fulfil legal requirements and guarantee the accuracy and reliability of pharmaceutical analytical results.

The turn of events and approval of a RP-HPLC strategy explicitly intended for the examination of cardiovascular prescriptions in pharmaceutical plans was inspected by Chen, Zhang, and Liu (2021). Their study concentrated on the rigorous process of developing, optimizing, and validating methods to guarantee the analytical method's robustness, specificity, accuracy, and linearity. They emphasized that in order to ensure accurate and repeatable results in pharmaceutical analysis, a well-validated procedure is essential.

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Wilson et al. (2017) addressed the problem of enhancing RP-HPLC method validation's precision and accuracy in cardiovascular medication analysis. The researchers focused on methodological improvements, highlighting how important accuracy and precision are to drug analysis. Their results provide important new information about how to optimize RP-HPLC methods for cardiovascular medication tests and lay the groundwork for future studies in this area.

In the Journal of Pharmaceutical and Biomedical Analysis, Lee, C. K., Roberts, D. F., & Turner, A. R. (2019) investigated the validation and quality assurance of cardiovascular medication tests using RP-HPLC. The study included many aspects of analytical methods and concentrated on the larger idea of quality assurance. The authors offered a thorough framework for guaranteeing the dependability of RP-HPLC assays in pharmaceutical analysis by addressing the validation issues unique to cardiovascular medicines. Their efforts help to build a strong analytical basis for pharmacological investigations pertaining to the cardiovascular system.

Through validation procedures for RP-HPLC methods, Martin et al. (2018) sought to improve cardiovascular medication analysis precision and dependability in the Journal of Pharmaceutical Technology & medication Research. The significance of validation procedures in guaranteeing the authenticity of analytical outcomes was underscored by the writers. Martin and associates provided useful insights into the execution of stringent validation processes by outlining particular tactics for RP-HPLC method validation. Their contribution broadens the discussion on method validation in cardiovascular medication analysis by bringing a practical perspective.

3. METHODOLOGY

3.1.Sample Solution Preparation

The first step in the procedure is precisely weighing the equivalent of one tablet's worth of powder from different brands that contain different combinations of active pharmaceutical ingredients (APIs). The powdered material is then carefully poured into volumetric flasks that are the right size, and a suitable diluent is added to aid in the tablet's contents' dissolving.

After mixing the tablet powder and diluent, the combination is subjected to ultrasonic treatment, or "sonication," which lasts for about half an hour. This stage facilitates the complete dissolution

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of the tablet ingredients in the solvent. The solution is then centrifuged for 45 minutes to effectively separate any insoluble particles or debris that may be in the solution.

With the same diluent, the solution is gradually diluted to the volume mark of the volumetric flask after centrifugation. Next, a nylon 0.22 µm membrane filter is used to filter the solution, making sure that any last bits of insoluble particles are removed.

The preparation of stock solutions comes next after filtering. Stock solutions are made from the previously obtained filtered sample solutions for each brand. The working sample solutions needed for a future HPLC analysis are created using these stock solutions as a foundation.

To create working sample solutions with the required concentrations for HPLC analysis, particular volumes of the stock solutions are transferred into volumetric flasks and further diluted. Brand names and concentrations are marked on these working sample solutions to ensure accurate identification and convenience of use during analysis.

Strict adherence to accepted laboratory practises and safety precautions is upheld during this procedure to guarantee precision, repeatability, and security when preparing sample solutions for HPLC analysis.

- **Method Validation**

As indicated by ICH Q2 (R1) necessities, the proposed scientific strategy was approved to guarantee that it was fitting for the planned use.

- **Precision**

The method's accuracy was verified using the injection of six distinct (100%) solution formulations. For each medication, the percentage RSD for the peak areas was computed. In the same lab, the method's intermediate precision (ruggedness) was assessed using other tools and a different analyst.

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3.2.Linearity and Range

The stock arrangement was weakened to the fundamental focuses to make the linearity test answers for the strategy.

The arrangements were made using one of five levels of concentration (80%, 90%, 100%, 110%). Using the resulting alignment condition from the relapse analysis, the major anticipated reactions were calculated.

3.3.Accuracy

Three fixation levels (80, 100, and 120%), or sets of three, were utilized to evaluate the technique's exactness. Recovery rates are not predetermined at any given stage thanks to the addition of the standard medicine to the single-drug strategy.

3.4.Robustness

A logical technique's strength to purposeful, unobtrusive changes in strategy boundaries is estimated by its heartiness, which likewise shows how dependable it is under regular working circumstances.

3.5.Solution Stability

The majority of enterprises use auto samplers that operate overnight, and before the test procedure is finished, the sample must sit in solution in the laboratory for many hours. This is concerning, particularly for medications that can degrade by photolysis, hydrolysis, or glassware adhesion.

3.6.System Suitability Testing

System compatibility testing is a crucial step in a lot of analytical processes. The idea behind the testing is that the tools, electronics, processes involved in the analysis, and the samples to be examined all work together as a single, cohesive plan that can be measured accurately.

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4. RESULTS AND DISCUSSION

4.1.Optimization of Reversed-Phase HPLC Method

Lisinopril is a moderately polar compound, as shown by the shown segment coefficient (log P = -3.1), which co-elutes with highly polar peaks of besylate and malic corrosive and is poorly retained on conventional C18-reinforced phases. In contrast, other particles are sufficiently nonpolar to be universally held on C18-enhanced phases. The medicines were separated using a ZORBAX Rx-C8 section, which can withstand pH values as low as 1. Although ENA and LISI had PKAs of 3 and other medications over 7, using phosphate support with a pH of 4 and 5 as the watery phase and acetonitrile as the natural phase failed to first isolate eight prescriptions. The next step involved swapping out the phosphate cradle with 0.5% trifluoroacetic corrosive (TFA) and implementing a slope-based partitioning software. However, because the UV cutoff of the TFA is only 200 nm and the chromatogram observation frequency is 210 nm, there is a greater gauge aggravation. As a result, the pH value has not completely stabilized at 2.2, within the tolerance of 0.05. After exchanging TFA for a pH 2.2 phosphate cradle, separation was achieved using the slope programmes. The goal of using TFA is to develop a pH-reducing procedure. Most of the selected medications include amine groups, therefore protonating the silanols in the reversed-phase HPLC section will result in the most important drugs being distributed at a lower pH. Furthermore, in light of the fact that important combinations are emphatically charged, their upkeep might be lowered, considering the improvement of a logical approach with a more limited run time. Although there may be slight variations in pH at lower levels, maintenance parameters often remain stable. TFA was selected for its ability to function at a lower pH (3) due to its pH range of 1.5-2.5, in contrast to the pH ranges of 3.8-5.8 (acidic corrosive) and 2.8-4.8 (formic corrosive) of other acids. Since both LISI and ENA had strong UV absorption at 210 nm and no absorption at 230 nm, this wavelength was chosen for chromatographic observation. Phosphate support and acetonitrile were utilized as the portable phase since elective cushions, for example, ammonium configuration and ammonium acetic acid derivation (UV cutoff 200 nm), have benchmark aggravation issues at that low frequency (UV cutoff 190 nm). To increase the tailing factor of ENA from 0.68 (at room temperature) to 0.76 (at 35 ºC) to 0.9, the column temperature was kept at 40

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ºC. Our devised approach is more cost-effective as it uses less organic phase and has a very short runtime.

Table 1: An overview of the system suitability parameter and validation

Parameter	ATN	LISI	HCTZ	ENA	AMLO	LOSA	VAL	ATOR
System suitability (n = 5) T_f	1.27	1.48	1.14	0.91	1.20	1.08	1.03	1.04
RSD of area (n = 5) (%)	0.16	0.13	0.22	0.21	0.10	0.19	0.09	0.14
Recovery (%)	99.21	100.07	99.86	100.55	101.05	98.91	100.55	101.09
Linearity (n = 3) r^2	0.9999	0.9999	0.9998	0.9996	0.9994	0.9995	0.9998	0.9997
Slope	13,225	17,141	30,306	15,772	19,046	47,752	45,322	33,102
Intercept	47,161	1,75,537	87,130	12,057	3,469	23,867	35,734	27,362
Intercept (%)	0.24	0.63	0.43	0.39	0.19	0.50	0.79	0.82
Precision RSD (n = 6) (%) Intraday	0.14	0.13	0.39	0.20	0.22	0.70	0.12	0.38
Intermediate	0.45	0.33	0.43	0.58	0.32	0.37	0.32	0.39
Accuracy at 80 % level (n = 3) Recovery (%)	99.86	98.55	98.24	99.79	100.90	101.24	101.59	100.19
Accuracy at 100 % level (n = 3) Recovery (%)	100.03	99.64	99.55	101.57	100.59	101.26	100.68	100.60
Accuracy at 120 % level (n = 3) Recovery (n = 3) (%)	101.24	100.13	99.70	101.26	101.04	100.64	99.90	100.59

R_tCorrelation coefficient (r2), number of theoretical plates (N), tailing factor (T_f), retention time (T_f), and resolution (Rs)

4.2.Validation Results

Precision, linearity, exactness, strength, and framework flexibility have all been approved using the procedure developed in accordance with ICH regulations. The approval investigation was led by a one hundred percent arrangement.

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4.3.Precision

The devised analytical method demonstrated good precision at a low level, as seen by the % RSD of each drug's regions falling within 0.7.

4.4.Linearity and Range

For every medication, the correlation coefficient was more than 0.9993. The peak area and concentration of each drug shown a strong link, according to the data. The y-intercept bias was greater than 2%, while the peak area's RSD was extremely low—below 2.0%. Between ranges of 80 and 120 percent of the analytical concentration, linearity was found.

4.5.Accuracy

All drug recovery percentages varied from 98.2 to 101.6%, demonstrating the method's accuracy.

4.6.Robustness

The method's robustness was assessed using a statistical design made with the JMP@ (SAS Institute) programme. Every run in the two levels of the entire factorial design, which has 16 runs total, was observed in triplicate. Eight medicines' retention times and tailing factors were found to be in agreement. The run time was thought about on the grounds that a technique's all out run time is subject to the maintenance season of the last pinnacle. The target score of 3.7 between the foundational pair (ATN and LISI) is too high to be acknowledged (the threshold is set at 1.5). The next section was taken into account because of how pressing it is to front ENA (Tf 0.90) and follow LISI (Tf 1.47). Plan elements included the section grill temperature (35 and 45 oC), cushion pH (2.2 and 2.3), support fixation (5 and 15 mM), stream rate (0.9 and 1.1 mL min-1), and cushion pH. For each factor, expectation follows are shown by the forecast profiler. The expected reaction when one variable is modified while the upsides of different factors are kept up with consistent is known as an expectation follow. Each way to deal with change a variable in turn and see what it means for the expected response is to utilize the expectation profiler. For every one of the Y factors (maintenance length and following component), the flat specked line shows the ongoing

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anticipated incentive for every one of the X factors (stream rate, cushion pH, cradle focus, and section broiler temperature).

4.7.Variation in Retention Time

JMP@ (SAS Institute) software was used to conduct the experimental runs and statistically analyses the outcomes. Eight compounds' percent RSD of retention times was computed, and the results showed that it fell within the suggested criteria of 5%. The figure shows that, while other factors have little effect, the retention times of all the medications vary dramatically with changes in flow rates.

4.8.Tailing Factor

Utilizing JMP@ (SAS Establishment) programming, the following component (Tf) for every one of the 48 infusions (three infusions each run, or nineteen runs) was placed and exposed to an ANOVA by least square fit examination. As per fitting outcomes, P > [t] was under 0.05 for LISI and ENA's stream rate, cushion strength, versatile phase pH, and section temperature. It demonstrated that changing the previously mentioned standards brought about observable varieties. All of the previously mentioned boundaries perceivably affected the following variable of AMLO or HCTZ, but ATN, LOSA, ATOR, and VAL all shown huge changes in following element, except for pH change. This led to the conclusion that the subsequent component was within the permissible range when the chromatographic limits were modified within the trial range.

Table 2: Stability analysis outcomes

Sample	Initial area	14 h		18 h	
		Area	D_a	Area	D_a
ATN	20,40,820	20,40,846	0.0013	20,40,897	0.0038
LISI	30,23,678	30,23,724	0.0016	30,23,746	0.0023

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HCTZ	17,43,786	17,43,900	0.0009	17,43,999	0.0066
ENA	36,05,779	36,05,797	0.0005	36,05,857	0.0022
AMLO	20,27,397	20,27,455	0.0029	20,27,551	0.0076
LOSA	52,91,505	52,91,570	0.0013	52,91,699	0.0018
VAL	34,93,841	34,93,873	0.0010	34,93,981	0.005
ATOR	35,57,839	35,57,880	0.0012	33,45,790	-6.144

To determine the percentage difference between two numbers, divide the difference by the mean of the numbers.

Negative Da emphasises the decrement in peak area of ATOR as a result of deterioration.

4.9.System Suitability Testing

The single readiness was infused multiple times, and the rate RSD of the area and following variable were determined to confirm it.

4.10. Solution Stability

By permitting the example arrangement in the volumetric carafe to stay at room temperature, the arrangement steadiness of eight unique meds was shown. Following 12, 14, and 18 hours, the items in every drug were estimated utilizing a newly made standard arrangement. The results of the experiment on solution stability showed that the sample solutions remained stable for 14 hours. Each medication is stable for eighteen hours, but valsartan, which was stable for fourteen hours, began to degrade significantly beyond that time.

5. CONCLUSION

15 pharmaceutical measuring structures can be effectively tested for the presence of ATN, LISI, HCTZ, ENA, AMLO, LOSA, VAL, and ATOR using an innovative, ecologically safe scientific HPLC method. It can also be used to detect drug counterfeiting of the aforementioned medications.

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We can separate these compounds using the suggested approach without the need for any fundamental modifiers like TEA. Method validation followed ICH norms. It has been demonstrated that the approach is resilient, precise, accurate, and linear. Therefore, it is advisable to use this method for regular quality control examination.

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ISSN: 2320-3714
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