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RESEARCH ARTICLE

Bivariate and Multivariate Calibration Approach for the Spectrophotometric Analysis of Two-Component Drugs in Real Tablets

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Bivariate and Multivariate Calibration Approach for the Spectrophotometric Analysis of Two-Component Drugs in Real Tablets

SUMMARY

This study presents a novel and simple spectrophotometric method for simultaneously quantifying rosuvastatin (ROS) and amlodipine (AML) in tablet formulation. Due to their overlapping absorption spectra, traditional spectrophotometric techniques were inadequate for analyzing these two drugs. To address this challenge, bivariate and multivariate calibration techniques were employed for the simultaneous determination of ROS and AML. Regression equations were generated using known concentrations and corresponding absorbance values of ROS and AML across different wavelengths, specifically between 224 and 371 nm. A total of 22 different regression equations were computed to establish the bivariate calibration method. The slope values from these equations were utilized to create sensitivity matrices, and the determinant of each sensitivity matrix was calculated for various wavelength pairs. An optimal wavelength pair was selected using Kaiser's technique, and a corresponding set of equations was constructed based on this chosen pair. The effectiveness of the proposed methods was validated through the analysis of synthetic mixtures and standard addition samples. Ultimately, the methods demonstrated a successful application for quantitatively analyzing ROS and AML in tablets.

Key Words: *Amlodipine, bivariate calibration method, multivariate calibration method, quantitative determination of tablets, rosuvastatin, spectrophotometric analysis.*

Gerçek Tabletlerdeki İki Bileşenli İlaçların Spektrofotometrik Analizi İçin İki Değişkenli ve Çok Değişkenli Kalibrasyon Yaklaşımı

ÖZ

Bu çalışma, tablet formülasyonunda rosuvastatin (ROS) ve amlodipin (AML) için eş zamanlı olarak tayin etmek için yeni ve basit bir spektrofotometrik yöntem sunmaktadır. Çakışan absorbans spektrumları nedeniyle, geleneksel spektrofotometrik teknikler bu iki ilacı analiz etmek için yetersizdir. Bu zorluğun üstesinden gelmek için, ROS ve AML'nin eş zamanlı tayini için iki değişkenli ve çok değişkenli kalibrasyon teknikleri kullanıldı. Regresyon denklemleri, bilinen konsantrasyonlar ve farklı dalga boylarında, özellikle 224 ile 371 nm arasında ROS ve AML'nin karşılık gelen absorbans değerleri kullanılarak oluşturuldu. İki değişkenli kalibrasyon yöntemini oluşturmak için toplam 22 farklı regresyon denklemi hesaplandı. Bu denklemlerden elde edilen eğim değerleri duyarlılık matrisleri oluşturmak için kullanıldı ve her duyarlılık matrisinin determinantı çeşitli dalga boyu çiftleri için hesaplandı. Kaiser tekniği kullanılarak optimum bir dalga boyu çifti seçildi ve bu seçilen çifte karşılık gelen bir denklem seti oluşturuldu. Önerilen yöntemlerin etkinliği, sentetik karışımların ve standart ekleme örneklerinin analizi aracılığıyla doğrulandı. Sonuç olarak, yöntemlerin tabletlerdeki ROS ve AML'nin kantitatif analizinde başarılı bir şekilde uygulanabileceği ortaya konmuştur.

Anahtar Kelimeler: *Amlodipin, çift değişkenli kalibrasyon yöntemi, çok değişkenli kalibrasyon yöntemi, tabletlerin kantitatif tayini, rosuvastatin, spektrofotometrik analiz.*

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INTRODUCTION

Cardiovascular diseases occupy a central place among global health problems and are the leading cause of death and morbidity worldwide (Roth, 2018). In recent years, national and international health systems and scientific research centers have been spending great financial and economic efforts on strategies to prevent the disease and manage risk factors in cases where diabetes, cholesterol, and hypertension coexist in individuals with cardiovascular disease. Most cardiovascular diseases can be prevented by implementing social strategies and controlling behavioral risk factors (such as unhealthy diet, tobacco use, obesity, physical inactivity, and harmful alcohol use) (World Health Organization, 2024). However, in cases where it cannot be prevented, patients must take more than one medication for treatment (Asia Pacific Cohort Studies Collaboration, 2005).

Multiple studies have shown that treating hypertension and dyslipidemia together with antihypertensive drugs and statins leads to a significant and synergistic reduction in cardiovascular risk, compared to managing these conditions separately (Multiple Risk Factor Intervention Trial Research Group,1982; Sever et al., 2003; Schwalm et al.,2016). The most recent European Guidelines recommend the combined use of angiotensin receptor blockers/angiotensin-converting enzyme inhibitors plus calcium channel blockers or thiazide-like diuretic drugs hypertension management. In these guidelines, amlodipine (AML) is recognized as one of the primary blood pressure-lowering drugs and is recommended for use together with statin group drugs in patients with coexistent dyslipidemia (Williams et al., 2018). Rosuvastatin (ROS) is a member of the statin class of drugs, that reduce cholesterol levels and that are effective in preventing cardiovascular diseases and lowering high cholesterol (Mccormick et al., 2000; Olsson et al., 2001). The combined use of AML and ROS drugs not only reduces blood pressure and lipid levels but also contributes to the reduction of cardiovascular events and mortality by treating patients in their complexity (Chapman, Yeaw & Roberts, 2010; Kim et al., 2020). Moreover, this combination, offered in a single pill formulation, reduces blood pressure and lipid values more effectively than two separate drugs (Kim et al., 2020; Sarzani et al., 2022). These clinical and pharmacological results require the development of accurate, sensitive, selective, and rapid analytical methods to achieve the co-determination of the binary combination drug in tablets. The literature review indicates that various liquid chromatographic methods, including HPLC-PDA (Ashfaq et al., 2014; Kansara et al., 2020; Saurabh & Nitin, 2013; Yılmaz & Yılmaz, 2020), and HPTLC (Kansara et al., 2020), are predominantly studied for the quantitative determination of AML and ROS in drug formulations or biological fluids. However, these methods need expensive and high-tech equipment and expertise. It is also important to note that spectrophotometric methods are widely used in literature for drug analysis due to their ease of use and inexpensiveness. On the other hand, overlapping absorption spectra of multiple drug substances make it difficult to use conventional spectrophotometric methods for drug combinations.

When analyzing multiple drug substances, overlapping spectra require more sophisticated models such as classical least-squares, inverse least-squares, partial least-squares, and principal component regression with commercially available software. In contrast, bivariate and multivariate calibration models are simple and powerful choices for the quantitative analysis of mixtures due to their simple mathematical treatments.

Bivariate calibration models are based on the selection of a suitable wavelength pair to get a calibration model, explaining the relationship between the absorbance values and the concentration of analytes using Kaiser's method to find the best sensitivity. On the other hand, the multivariate calibration method of regression equations is constructed using n-wavelengths without needing a choice of suitable wavelength pair for quantifying analytes in a mixture. Details of theoretical principles and application of the bivariate and multivariate calibration models based on the regression equations to analyze a two-component mixture can be found in Dinç, 2003.

In this work, the bivariate and multivariate calibration approaches were proposed to resolve the overlapping spectra and quantify ROS and AML in commercial tablets without a preliminary separation step.

MATERIAL AND METHODS

Instruments and software

A Shimadzu UV-160 double-beam UV-Vis spectrophotometer with Shimadzu UVPC software was used to procure the absorption spectra of the compounds and their samples in the spectral range of 200-420 nm. MATLAB 7.0 and Microsoft Excel were used for data acquisition and statistical calculations.

Chemicals and reagents

The drug standards, rosuvastatin calcium (≥98%) and amlodipine besylate (≥98%) were obtained from Sigma-Aldrich (Steinheim, Germany). For spectrophotometric analysis, the methanol, which is of HPLC grade, was used as a solvent and purchased from J.T. Baker (Netherlands). All solutions were freshly prepared daily and kept in the dark throughout the analysis to maintain the stability.

Preparation of stock standard, calibration, and validation solutions

To prepare stock solutions of ROS and AML, 0.01 grams of each compound were separately weighed and dissolved in methanol in 100 mL volumetric flasks. These stock solutions were then appropriately diluted with methanol to get calibration, and validation sample solutions would be checked with the bivariate and multivariate calibration techniques.

To apply the bivariate and multivariate calibration approaches, standard calibration solutions were prepared in the concentration range of 4.0-28.0 µg/mL for ROS and AML. The 11 synthetic mixtures containing ROS and AML in different levels within the working concentration range were prepared similarly. The standard addition samples were prepared in triplicates by adding AML and ROS standard solutions (0, 6, 14, and 22 µg/ mL) to a portion of the commercial tablet sample solution.

Preparation of Tablet Samples

Ten tablets of ROSUCOR PLUS® (Celtis İlaç Ltd. Şti.) were accurately weighed, and the quantity corresponding to one tablet (266.3 mg) was transferred to a 100 mL volumetric flask after thorough pulverization of the tablets in a mortar. The flask was filled with methanol, and the powdered sample was mixed with a mechanical stirrer for 30 minutes. Filtration was conducted through a 0.45 µm pore size filter (Pall Industries, USA). The filtrate was diluted with methanol (1:10) to reach a concentration of 20 µg/ mL ROS and 10 µg/ mL AML. The diluted tablet solution was subjected to UV-VIS analysis for the application of the bivariate and multivariate calibration approaches. The declared contents of the tablet were as follows: 20 mg ROS and 10 mg AML per tablet.

RESULTS AND DISCUSSION

In traditional spectral analysis methods, the most common handicap is the overlapping of spectral bands in the solution of two or more components. To overcome this difficulty in the analysis, we applied bivariate and multivariate calibration methods which are reliable, simple, cheap, and rapid to the quantitative simultaneous determination of ROS and AML. The bivariate approach uses the four linear regression calibration equations with two calibrations for each component at two wavelengths selected (Demirbilek, Dinç & Baleanu, 2010; Dinç, Arslan & Baleanu, 2008).

Standard solutions of ROS and AML were prepared within a working concentration range of 4.0 to 28.0 µg/mL. The absorption spectra of these sample solutions were recorded from 200 to 420 nm, as shown in Figure 1.

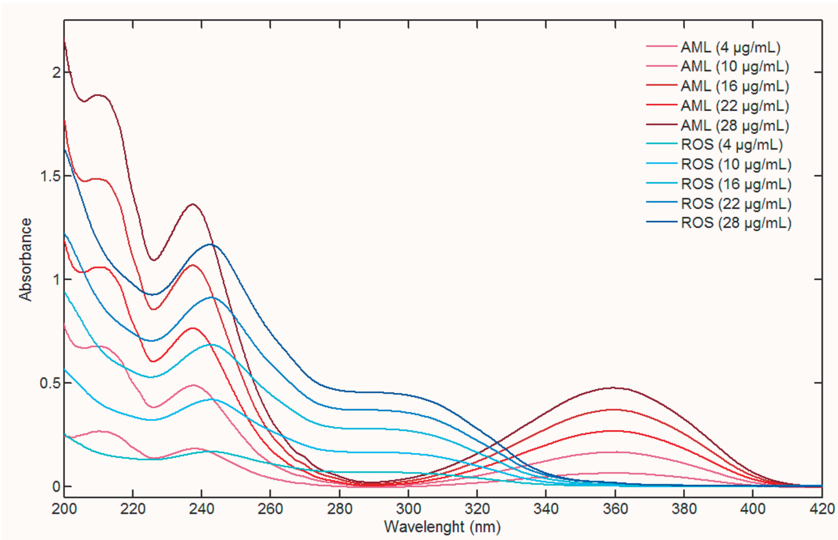

Figure 1. Absorbance spectra of ROS and AML in the working concentration range of 4.0-28.0 µg/mL

The traditional spectrophotometric method could not analyze these two drugs in their mixtures because their spectra overlap. Bivariate and multivariate calibration techniques with simple mathematical algorithms and applications were developed to simultaneously determine both ROS and AML in the same binary mixtures. To build bivariate and multivariate calibration methods, we computed 22 different regression equations using known concentrations and absorbance values across various wavelengths ranging from 224 nm to 371 nm. The slope values derived from these equations were used to create sensitivity matrices. We calculated the determinants of the sensitivity matrices for each pair of wavelengths, which are illustrated in Figure 2.

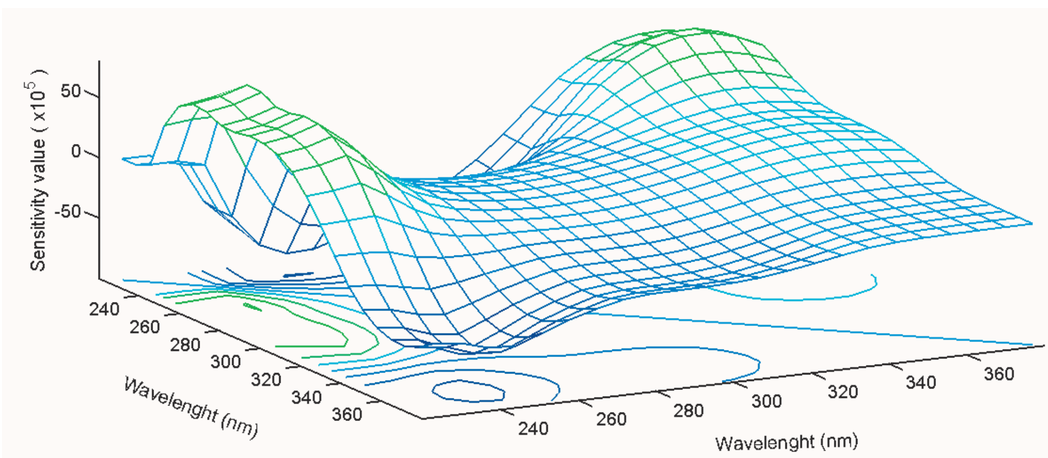

Figure 2. Three-dimensional illustration of wavelength pairs and corresponding sensitivity values.

For the bivariate calibration, an optimal pair of wavelengths were selected as 238 nm and 259 nm using Kaiser's technique. Then, an equation set was constructed using this wavelength pair to implement a bivariate calibration approach.

Unknown concentrations of AML and ROS were obtained by solving the equations constructed using the bivariate approach. Using the optimal wavelength pair, the bivariate equation system was obtained. This linear equation system in matrix format is represented below:

$$\begin{pmatrix} A_{mix_{238nm}} \\ A_{mix_{259nm}} \end{pmatrix} = \begin{pmatrix} 4.88x10^{-2} & 4.02x10^{-2} \\ 1.30x10^{-2} & 2.73x10^{-2} \end{pmatrix} x \begin{pmatrix} C_{AML} \\ C_{ROS} \end{pmatrix} + \begin{pmatrix} -4.57x10^{-3} \\ 5.27x10^{-3} \end{pmatrix}$$

The concentrations of ROS and AML in the analyzed synthetic mixtures were quantified using the linear equation above.

The multivariate calibration approach is similar to the bivariate method; however, it utilizes multiple wavelengths instead of just two [26]. In this case, a multivariate calibration technique was employed using all 22 regression equations at critical points, including the maximum, shoulder, and minimum values within the spectral range of 224-278 nm for the related compounds in the binary mixtures. The system of the 22 regression equations was represented as follows:

$$\begin{pmatrix} A_{\text{ü}\ \text{ü}\ nm} \\ A_{\text{ü}\ \text{ü}\ nm} \\ \text{ü} \\ \text{ü} \\ A_{\text{ü}\ \text{ü}\ nm} \end{pmatrix} = \begin{pmatrix} 4.16\text{ü}10^{\text{Ni}} & 3.31\ \ 10 \\ 4.39\text{ü}10^{\text{Ni}} & 3.49\ \ 10 \\ \ldots & \ldots \\ \text{ü} & \ldots \\ 1.27x10^{\text{Ni}} & 2.55x10 \end{pmatrix} x \begin{pmatrix} C_{\text{ü}} \\ C_{\text{ü}} \end{pmatrix} + \begin{pmatrix} -4.52\ \ 10 \\ -2.05\ \ 10 \\ \ldots \\ \ldots \\ -4.04x10 \end{pmatrix}$$

Validation studies were performed using synthetic samples, and standard addition samples. The results of recovery studies as percentage average recoveries and their relative standard deviations for both bivariate and multivariate calibration methods are given in Table 1. The results were satisfactory with appropriate accuracy and precision without requiring preliminary separation step.

Table 1. Recovery results of synthetic test samples

			Bivariate		Multivariate		Bivariate		Multivariate	
	Added (µg/µL)		Found		Found		Recovery (%)		Recovery (%)	
No.	AML	ROS	AML	ROS	AML	ROS	AML	ROS	AML	ROS
1	4	20	4.07	19.98	4.07	19.98	101.6	99.9	101.8	99.9
2	10	20	10.62	19.20	10.32	19.83	106.2	96.0	103.2	99.1
3	16	20	16.56	19.12	16.24	19.71	103.5	95.6	101.5	98.6
4	22	20	22.34	18.86	21.93	19.52	101.5	94.3	99.7	97.6
5	28	20	28.27	19.20	27.99	19.77	101.0	96.0	100.0	98.8
6	10	4	10.35	3.97	10.69	3.94	103.5	99.2	106.9	98.4
7	10	10	10.50	9.95	10.04	10.22	105.0	99.5	100.4	102.2
8	10	16	10.32	15.60	9.91	16.44	103.2	97.5	99.1	102.8
9	10	22	10.61	21.50	10.36	22.08	106.1	97.7	103.6	100.4
10	10	28	10.63	26.59	10.23	27.32	106.3	95.0	102.3	97.6
11	10	20	10.60	20.46	10.62	20.68	106.0	102.3	106.2	103.4
						Mean	104.0	97.3	102.2	99.8
				Standard deviation			1.94	1.95	2.45	1.76
				Relative standard deviation			1.87	2.01	2.40	1.76

Standard addition samples were prepared at three distinct concentration levels for ROS and AML (6, 14, and 22 μg/mL) and analyzed with the two proposed calibration methods. Then added recovery results for each drug were calculated. Their results (recovery value and standard deviation) are presented in Table 2. The experimental results were calculated as the mean of triplicate measurements for each concentration level. As evidenced by the results presented in Table 2, the composition of the sample matrix did not influence the analysis of simultaneous AML and ROS. In other words, the results were found to be satisfactory for the selectivity of the proposed analytical methods.

Table 2. Quantitative results of standard addition samples

		Added (μg/μL)		Found (μg/μL)			
				Bivariate calibration		Multivariate calibration	
		AML	ROS	AML	ROS	AML	ROS
Tablet	+	6	6	6.06	5.83	5.76	6.00
Tablet	+	14	14	14.41	9.96	14.06	10.09
Tablet	+	22	22	22.88	14.47	22.21	14.94
		Recovery (%)		102.6	100.0	99.1	102.5
		RSD		0.33	0.36	0.28	0.39

RSD: Relative standard deviation

Bivariate and multivariate calibration methods were used to quantify the active drugs, AML and ROS, simultaneously in real tablet samples. The experimental results, which were calculated as an average of ten measurements (n=10) for real tablet analysis, presented in Table 3, demonstrate a high degree of agreement with the labeled claims of the tablet samples. Additionally, the standard deviation and relative standard deviation values provided in Table 3 confirm the reliability and effectiveness of the proposed methods.

Table 3. Assay results of the commercial tablets.

	Bivariate calibration		Multivariate calibration	
	AML	ROS	AML	ROS
Mean*	10.10	19.60	9.96	20.08
Standard deviation	0.13	0.45	0.11	0.31
Relative standard deviation	1.32	2.30	1.13	1.52

* n = 10
(Label claim: 10 mg AML/20 mg ROS per tablet)

CONCLUSION

Two simple mathematical models, bivariate and multivariate calibration approaches based on the use of linear regression functions, were proposed to quantify ROS and AML in binary mixtures and commercial tables without a preliminary separation procedure. Both methods gave suitable results, although multivariate calibration results were slightly better (in terms of closeness to the label claim and standard deviation) When comparing the spectral bivariate and multivariate approaches with the HPLC method given in the literature (Ashfaq et al.), the proposed spectral methods have some advantages, such as simplicity, rapidity, ease of application, and cost-effectiveness for the quality control and routine analysis of tablets containing ROS and AML. The mentioned HPLC analysis requires expensive instrumentation, a long analysis period (e.g., long runtime, more than 20 minutes, to analyze ROS and AML), excessive solvent conception, etc. These methods can be easily applied in routine quality control. Once the bivariate and multivariate calibration equations are constructed, the concentration of analytes in unknown solutions can be easily calculated from the absorbance values by solving these equations. Consequently, the bivariate and multivariate calibrations provided new and alternative ways to quantitatively resolve the mixtures containing the analyzed drugs with short analysis time and low cost. Assay results showed that these methods were fast, easy, cheap, and suitable for the routine analysis and quality control of tablets containing AML and ROS.

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AUTHOR CONTRIBUTION STATEMENT

Conceptualization, Formal Analysis, Resources, Supervision, Writing-Review & Editing (ED), Investigation, Data curation, Writing-Original Draft, Writing-Review & Editing (AÜ), Data curation, Writing-Original Draft, Writing-Review & Editing (ZCE), Investigation (ÖFG)

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

REFERENCES

Ashfaq, M., Akhtar, T., Mustafa, G., Danish, M., Razzaq, S.N., & Nazar, M.F. (2014). Simultaneous estimation of rosuvastatin and amlodipine in pharmaceutical formulations using stability indicating HPLC method, *Brazilian Journal of Pharmaceutical Sciences*, *50*(3), 629-638. http://dx.doi.org/10.1590/S1984-82502014000300023

Asia Pacific Cohort Studies Collaboration. (2005). Joint effects of systolic blood pressure and serum cholesterol on cardiovascular disease in the Asia Pacific region. *Circulation*, *112*(22), 3384-3390. doi:10.1161/circulationaha.105.537472

Banerjee, S.K., & Vasava, N.M. (2013). Simultaneous estimation of amlodipine and rosuvastatin in combined bulk forms by RP-HPLC using ultraviolet detection, *Bulletin of Pharmaceutical Research. 3*(1), 29-33. Retrieved from https://journal.appconnect.in/

Chapman, R.H., Yeaw, J., & Roberts, C.S. (2010). Association between adherence to calcium-channel blocker and statin medications and likelihood of cardiovascular events among US managed care enrollees. *BMC Cardiovascular Disorders, 10,* 29. doi:10.1186/1471-2261-10-29

Demirbilek, M.P., Dinç, E., & Baleanu, D. (2010). Spectrophotometric Simultaneous Determination of Atorvastatin-Amlodipin and Telmisartan-Hidrochlorothiazide Mixtures by Bivariate and Multivariate Calibrations, *Revista de Chimie*, *61*(6), 532-540. Retrived from https://revistadechimie.ro/

Dinç, E. (2003). Linear regression analysis and its application to the multivariate spectral calibrations for the multiresolution of a ternary mixture of caffeine, paracetamol and metamizol in tablets, *Journal of Pharmaceutical and Biomedical Analysis, 33,* 605-615. https://doi.org/10.1016/S0731-7085(03)00260-7

Dinç, E., Arslan, F., & Baleanu, D. (2008). Spectrophotometric simultaneous Analysis of two-component mixture by bivariate and multivariate calibrations using the linear regression functions, *Revue Roumaine de Chimie*, *53*(8), 607-615. Retrived from https://revroum.lew.ro/

Kansara, D., Chhalotiya, U.K., Kachhiya, H.M., & Patel, I. (2020). Development of TLC method for simultaneous estimation of novel combination of amlodipine besylate, rosuvastatin calcium, and fimasartan potassium in synthetic mixture, *Journal of Chemical Metrology, 14*(2), 142-152. http://doi.org/10.25135/jcm.45.20.07.1744

Kansara, D.A., Chhalotiya, U.K., Kachhiya, H.M., Patel, I.M., & Shah, D.A. (2020). Simultaneous estimation of amlodipine besylate, Rosuvastatin calcium and Fimasartan potassium trihydrate combination used in the treatment of hypertension using LC method, *SN Applied Sciences, 2,* 948. https://doi.org/10.1007/s42452-020-2758-4

Kim, W., Chang, K., Cho, E.J., Ahn, J.C., Yu, C.W., Cho, K.I., …Park, C.G. (2020). A randomized, double-blind clinical trial to evaluate the efficacy and safety of a fixed-dose combination of amlodipine/rosuvastatin in patients with dyslipidemia and hypertension. *The Journal of Clinical Hypertension, 22*(2), 261-269. doi:10.1111/jch.13774.

Mccormick, A.D., Mckillop, D., Butters, C.J., Miles, G.S., Baba, T., & Touchi, A. (2000). ZD4522-an HMG-CoA reductase inhibitor free of metabolically mediated drug interactions: metabolic studies in human in vitro systems. *The Journal of Clinical Pharmacology*, *40,* 1055.

Multiple Risk Factor Intervention Trial Research Group (1982). Multiple risk factor intervention trial. Risk factor changes and mortality results. *JAMA*. *248*(12), 1465-77. doi:10.1001/jama.1982.03330120023025

Narapusetti, A., Bethanabhatla, S.S., Sockalingam, A., Repaka, N., & Saritha, V. (2015). Simultaneous determination of rosuvastatin and amlodipine in human plasma using tandem mass spectrometry: Application to disposition kinetics, *Journal of Advanced Research, 6*(6), 931-940. doi: 10.1016/j.jare.2014.08.010

Olsson, A.G., Pears, J., Mckellar, J., Mizan, J., & Raza, A. (2001). Effect of rosuvastatin on low-density lipoprotein cholesterol in patients with hypercholesterolemia. *American Journal of Cardiology, 88,* 504-508.

Roth, G. (2018). GBD 2017 Causes of Death Collaborators. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet*. *392*(10159), 1736-88. doi:10.1016/S0140-6736(18)32203-7.

Sarzani, R., Laureti, G., Gezzi, A., Spannella, F., & Giulietti, F. (2022). Single-pill fixed-dose drug combinations to reduce blood pressure: the right pill for the right patient. *Therapeutic Advances in Chronic Disease*. *24*(13). doi:20406223221102754.

Schwalm, J.D., McKee, M., Huffman, M.D., & Yusuf, S. (2016). Resource effective strategies to prevent and treat cardiovascular disease. *Circulation, 133*(8), 742-55. doi: 10.1161/CIRCULATIONAHA.115.008721.

Sever, P.S., Dahlöf, B., Poulter, N.R., Wedel, H., Beevers, G., Caulfield, M., … Ostergren, J. (2003). Prevention of coronary and stroke events with atorvastatin in hypertensive patients who have average or lower-than-average cholesterol concentrations, in the Anglo-Scandinavian Cardiac Outcomes Trial-Lipid Lowering Arm (ASCOT-LLA): A multicentre randomised controlled trial. *The Lancet, 361*(9364), 1149–1158. doi:10.1016/S0140-6736(03)12948-0

Williams, B., Mancia, G., Spiering, W., Agabiti, R. E., Azizi, M., Burnier, M., …. Desormais, I. (2018). 2018 ESC/ESH guidelines for the management of arterial hypertension. *European Heart Journal, 39,* 3021-3104. doi:10.1093/eurheartj/ehy339.

World Health Organization. (2017). Cardiovascular diseases fact sheet. Retrieved December 25, 2024, from https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds)

Yılmaz, B., &Yılmaz, N. (2020). Simultaneous determination of rosuvastatin and amlodipine in binary mixtures by differential pulse voltammetry and HPLC methods, *Eurasian Chemical Communications*, *2*(8), 881-894. doi:10.33945/sami/ecc/ecc.2020.228276.1045