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Comprehensive Evaluation and Stability Indication of HPLC-UV and HPLC-Fl Methods for the Synchronic Analysis of Three Antihypertension Agents Hydrochlorothiazide, Amlodipine, and Valsartan

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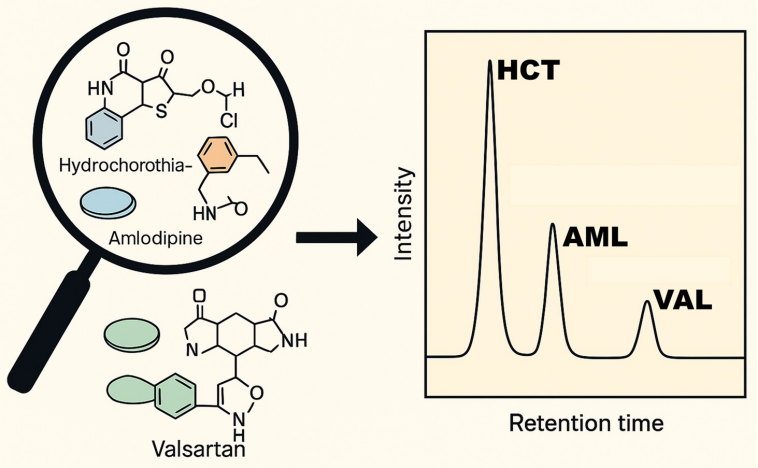
Abstract

Simultaneous determination of hydrochlorothiazide (HCT), amlodipine (Am), and valsartan (Val) drugs is performed. A high-performance liquid chromatography (HPLC) method with UV detection is used at 315 nm for the determination of hydrochlorothiazide, 357 nm for amlodipine, and 245 nm for valsartan. The method is applied in solution and pharmaceutical formulations. The method was extended to detect the three drugs by HPLC with a fluorescence detector at (λ_{ex} = 315/λ_{em} = 321 nm for hydrochlorothiazide, λ_{ex} = 357/λ_{em} = 365 nm for amlodipine, and λ_{ex} = 245/λ_{em} = 247 nm for valsartan). The HPLC/UV and HPLC/Fl methods were found to be precise, accurate, and sensitive. Gradient separation was employed on a C18 column (250 × 4.6 mm i.d., 5 µm) at room temperature. A water/acetonitrile/glacial acetic acid mixture (300:700:1 by volume) was used as the mobile phase. The drugs under investigation were found to have 93% - 101% recovery from solution and pharmaceutical formulations. The calibration curve was linear over the range of 0.010 - 10.0 µg/mL for hydrochlorothiazide, amlodipine, and valsartan. The limits of quantification (LOQ) were: (0.020 µg/mL) for hydrochlorothiazide, (0.030 µg/mL) for amlodipine, and (0.030 µg/mL) for valsartan. The limits of detection (LOD) were: 0.010, 0.020 µg/mL, and 0.020 µg/mL for hydrochlorothiazide, amlodipine, and valsartan, respectively.

Graphical Abstract



Keywords

Hydrochlorothiazide, Amlodipine, Valsartan, Combination, Drug Analysis

1. Introduction

High blood pressure (hypertension) leads to the disability of heart function. When it continues for a long time, the heart and arteries function improperly, and this leads to a deadly heart attack. In the past, amlodipine, valsartan, and hydrochlorothiazide medicines (Figure 1) were used separately to treat hypertension. Hydrochlorothiazide is used as a diuretic (water pill), amlodipine is a calcium channel blocker (CCB), and valsartan is an angiotensin II receptor blocker (ARB). The separate use of these medicines was not highly efficient. As a result, a combination of hypertension medicines is applied. The simultaneous use of double or triple combinations of Aml/Val/HCT was found to be very efficient in controlling hypertension.

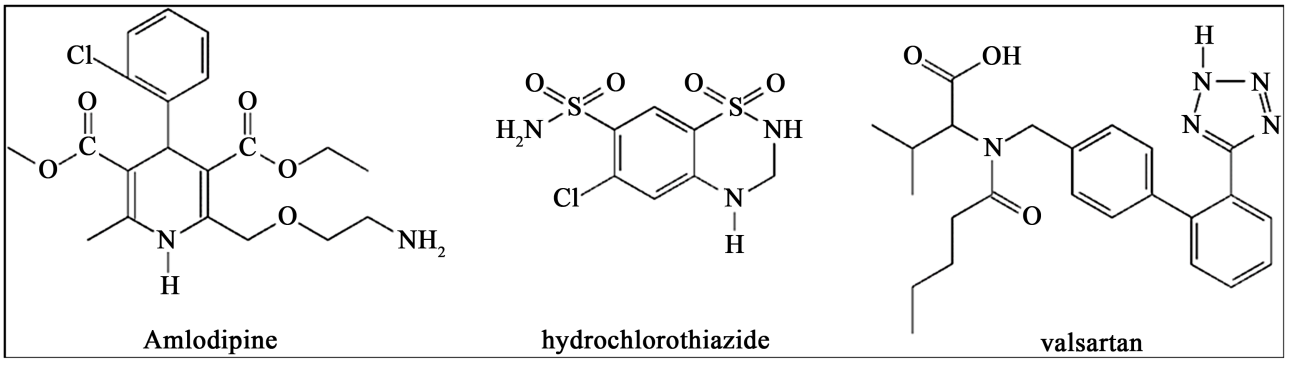


Figure 1. The chemical structure of amlodipine (Aml), hydrochlorothiazide (HCT), and valsartan (Val).

Many studies have been conducted over the recent decades to develop techniques for the detection of amlodipine, valsartan, and hydrochlorothiazide sepa-

rately or together.

The three drugs have been analyzed by liquid chromatography-ultraviolet spectroscopy [1] [2], liquid chromatography-mass spectroscopy [3] [4], liquid chromatography-fluorescence detection, spectrophotometric determination [5], HPLC/DAD [6], spectrofluorometric determination [7], and others. Moreover, the three drugs have been analyzed in plasma and urine samples by different chromatographic and spectroscopic methods such as liquid chromatography-mass spectroscopy [8], liquid chromatography-ultraviolet spectroscopy, and liquid chromatography-fluorescence detection [9].

2. Experimental Part

2.1. Materials and Methods

The HCT, Am, and Val standards (>99% purity) were purchased from Sigma-Aldrich and used without further purification. Acetonitrile and methanol solvents (HPLC grade) were purchased from Merck. Distilled water was used to prepare the standards and mobile phase. A UV/Vis spectrophotometer (type Shimadzu UV-1800 UV-VIS) with a 1.0 cm cuvette was used to perform the UV-Vis scans for HCT, Am, and Val. The chromatographic separations were performed using the Shimadzu I-Series LC-2030 (HPLC) with UV-VIS/fluorescence detectors.

2.2. Standard and Working Solutions

Standard stock solutions (10 μg/ml) of HCT, Am, and Val were prepared by dissolving 10 mg of each in 25 mL of methanol. The standard solutions were scanned over the range of 200 - 800 nm to detect the maximum wavelengths. λ_{max} of 315 nm is observed for the determination of hydrochlorothiazide, 357 nm for amlodipine, and 245 nm for valsartan. Volumes of stock solution of HCT, Am, and Val were diluted separately to get a series of solutions containing 0.040 - 1.50 μg/mL for hydrochlorothiazide, 0.040 - 1.50 μg/mL for amlodipine, and 0.040 - 1.50 μg/mL for valsartan. Moreover, volumes of stock solutions of HCT, Am, and Val were combined and diluted to get a series of solutions containing 0.040 - 1.50 μg/mL for hydrochlorothiazide, amlodipine, and valsartan.

A formulated tablet of HCT, Am, and Val was purchased, and 10 tablets were ground, milled, and mixed homogeneously and inserted into a 500 mL volumetric flask. The powder was mixed with 250 mL of methanol, and the mixture was exposed to an ultrasonicating device for 15 min. The volume was made up to 500 mL with methanol. All solutions were stored in a refrigerator at a temperature of 5˚C \u1d3c.

3. Results and Discussion

Using a UV-Vis spectrophotometer, it was found that the maximum wavelengths (λ_{max}) at which solutions have the highest absorbance in the spectrum for HCT, Am, and Val were found to be 315, 357, and 245 nm, respectively. These $\lambda_{max's}$ were selected to build the calibration curve and perform all absorbance measurements.

Also, these $\lambda_{max's}$ were used as λ_{ex} in the HPLC fluorescence measurements. Validation of the developed methods was carried out under the guidelines of the International Conference on Harmonization ICH.

3.1. Linearity and Calibration Curves

The calibration standards are introduced to the HPLC system twice: the first when a UV detector is connected to the HPLC, and the second when a fluorescence detector is connected to the HPLC (chromatograms are shown in Figure 2). The calibration curves were made by plotting the concentration of HCT, Am, and Val (x-axis) and their peak areas (y-axis). The calibration graphs of HCT, Am, and Val were shown in Figure 3 and Figure 4 for UV and fluorescence measurements, respectively. The graphs show: the correlation coefficients (r^2), the slopes, the intercepts, and the straight-line equations for each drug.

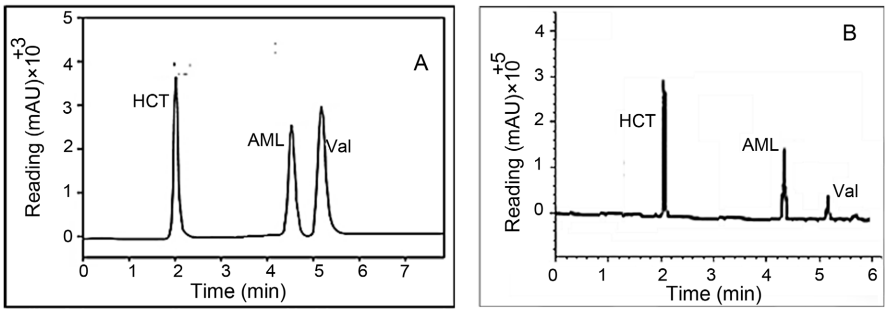


Figure 2. HPLC chromatogram of HCT/AML/VAL using: (A) UV detector, (B) Fluorescence detector.

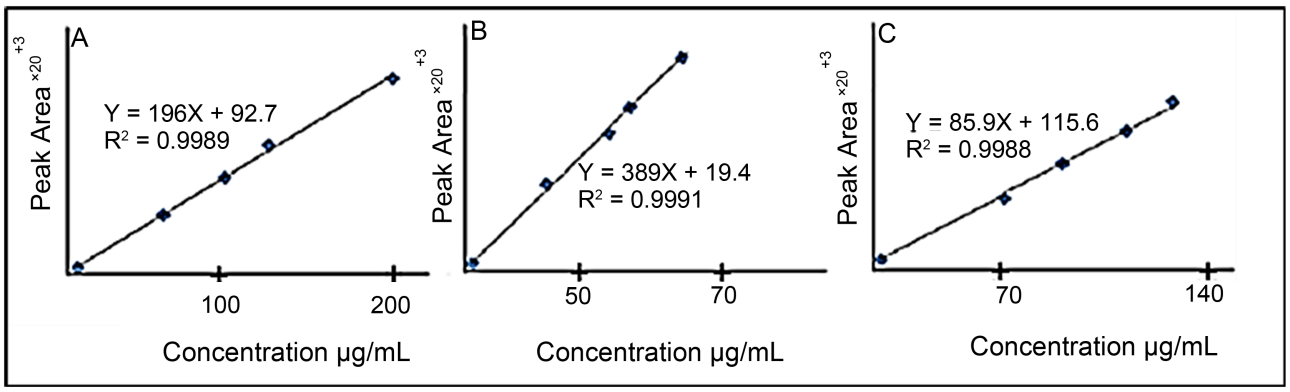


Figure 3. Calibration curves for the three drugs: (A) HCT, (B) Am, (C) Val. Results of HPLC/UV detector.

Seven calibration curves for each drug were prepared and injected into the HPLC with a UV detector. Each calibration curve has a straight-line equation with a correlation coefficient (r^2), a slope, and an intercept. Based on the results of the linearity studies, the developed method was set to be linear within the specified range. The (Absorbance/Concentration) relationship meets the analytical acceptance criteria and can be used for a sample containing an unknown quantity within an accepted confidence interval. The linearity parameters for HPLC/UV are illus-

trated in Table 1. The same standards that were used to build the calibration curves for the HPLC/UV data were injected again after changing the detector to fluorescence. The linearity parameters for HPLC/Fl are illustrated in Table 2.

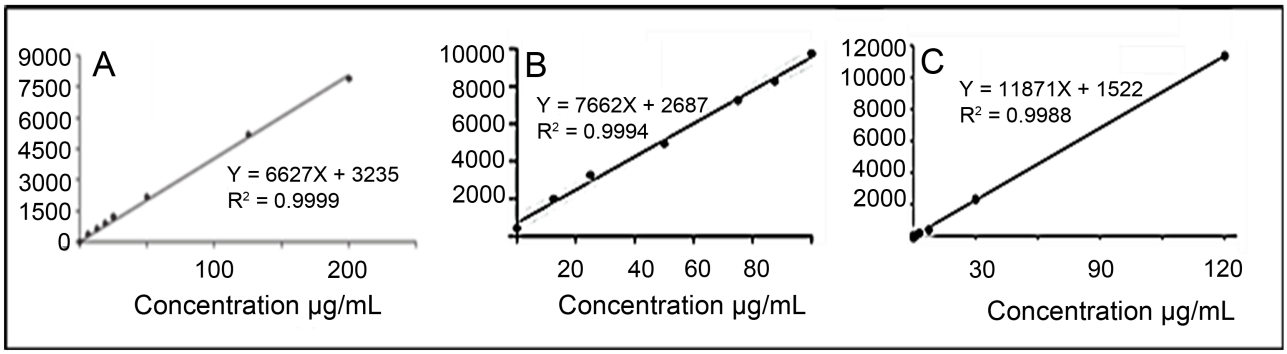


Figure 4. Calibration curves for the three drugs: (A) HCT, (B) Am, (C) Val. Results of HPLC/Fl detector.

Table 1. Calibration curves and linearity parameters as a result of the HPLC/UV detector.

Drug	HCT			Am			Val		
Curve	Slope	Intercept	Correlation Coefficient R^2	Slope	Intercept	Correlation Coefficient R^2	Slope	Intercept	Correlation Coefficient R^2
Curve 1	196	92.7	0.9989	389	19.4	0.9991	85.9	115.6	0.9993
Curve 2	203	95.1	0.9991	397	21.1	0.9990	91.3	111.5	0.9987
Curve 3	177	87.0	0.9990	376	19.6	0.9989	98.2	118.4	0.9990
Curve 4	212	90.3	0.9987	403	22.0	0.9990	75.7	115.1	0.9999
Curve 5	189	99.6	0.9985	401	18.9	0.9981	85.0	115.9	0.9987
Curve 6	190	78.5	0.9989	388	19.5	0.9987	81.4	108.7	0.9982
Curve 7	202	88.9	0.9992	392	18.7	0.9985	75.1	112.2	0.9997

Table 2. Calibration curves and linearity parameters as a result of HPLC/Fl detector.

Drug	HCT			Am			Val		
Curve	Slope	Intercept	Correlation Coefficient R^2	Slope	Intercept	Correlation Coefficient R^2	Slope	Intercept	Correlation Coefficient R^2
Curve 1	61,627	102,353	0.9999	76,624	26,875	0.9994	1,218,712	15,223	0.9999
Curve 2	65,331	92,400	0.9951	61,420	33,135	0.9991	1,001,615	11,628	0.9980
Curve 3	62,677	90,122	0.9980	73,313	20,344	0.9999	973,914	13,783	0.9995
Curve 4	60,125	107,451	0.9993	80,831	29,725	0.9939	1,331,013	11,527	0.9979
Curve 5	55,438	80,321	0.9928	70,872	21,513	0.9944	919,916	10,392	0.9991
Curve 6	71,702	77,550	0.9990	71,690	25,795	0.9987	989,913	19,096	0.9993
Curve 7	58,530	89,794	0.9898	69,384	31,002	0.9989	1,112,552	12,314	0.9988

3.2. Specificity

This test is investigated by observing any chromatographic interference of one drug with the other two drugs. The test was also evaluated for the interference of the excipients in the tablet solution with the drugs.

3.3. Accuracy

Accuracy is the ability of the method to produce results close to the true value. Quality control (QC) samples and a calibration curve are injected into the HPLC system. Back calculation is applied, and the real concentration of the QC is obtained from the straight-line equation. The obtained concentration is compared to the prepared concentration. The developed method was found to be valid and accurate since the relative errors are within the acceptable range (Table 3). The tablet solution was tested, taking into consideration that the prepared QC is the recorded dose on the tablet bar.

Table 3. Parameters of the accuracy data of the used QCs.

Method Accuracy				
Drug	Quality Control Conc. (μg/mL)	Mean Determined Concentration (n = 6)	Accuracy	
			Bias %	Relative Error %
Hydrochlorothiazide (HCT)	0.040	0.039	−0.002	−5.114
	0.015	0.014	−0.012	−5.173
	0.500	0.485	−0.033	−1.119
	1.500	1.508	0.016	0.388
Amlodipine (Am)	0.040	0.037	−0.002	−4.811
	0.015	0.013	−0.013	−4.977
	0.500	0.477	−0.032	−1.029
	1.500	1.498	−0.002	0.309
Valsartan (Val)	0.040	0.038	−0.002	−5.133
	0.015	0.014	−0.014	−4.989
	0.500	0.481	−0.036	−1.013
	1.500	1.501	0.013	0.375

3.4. Precision

In this part, the developed method was tested to determine whether it has high repeatability or not. Precision was measured for quality controls with low, medium, and high concentrations. Since the drug peak area is good evidence for repeatability, there was no need to introduce a calibration. Standard deviation (SD) and coefficient of variation (CV) of 6 replicates of each QC were calculated. The developed method was found to be precise since the SD and CV values are within

the acceptable range (Table 4). The tablet solutions were tested. The drug peak areas in the tablet solution were compared to QCs prepared with the exact concentration as the recorded dose on the tablet bar.

Table 4. Parameters of precision data of the used QCs.

Method Precision				
Drug	Quality Control Conc. (μg/mL)	Mean Determined Concentration (n = 6)	SD %	CV %
Hydrochlorothiazide (HCT)	0.040	0.041	0.005	2.963
	0.015	0.015	0.021	3.011
	0.500	0.494	0.022	2.368
	1.500	1.510	0.057	2.391
Amlodipine (Am)	0.040	0.043	0.004	1.993
	0.015	0.014	0.023	3.301
	0.500	0.497	0.023	2.176
	1.500	1.513	0.065	2.925
Valsartan (Val)	0.040	0.039	0.004	3.163
	0.015	0.016	0.027	2.991
	0.500	0.502	0.021	2.767
	1.500	1.509	0.059	2.238

3.5. LOD and LOQ

The limit of detection (LOD) of a method is the lowest amount of analyte in a sample that can be detected but not necessarily accurately quantified. The LOD is determined by the following equation: LOD = (3.3 σ)/S, where σ is the standard deviation of 6 replicates of blank response and S is the slope of the calibration curve. The limit of quantification (LOQ) is the lowest amount of analyte in a sample that can be accurately detected and quantified. The LOQ is determined by the following equation: LOQ = (10 σ)/S, where σ is the standard deviation of 6 replicates of blank response and S is the slope of the calibration curve.

3.6. Robustness and ruggedness

Degradation of the three drugs was tested under different stress conditions, namely: basic conditions, acidic conditions, long-term storage conditions, and storage temperature.

3.7. Basic Conditions

Sodium hydroxide (NaOH) (1.0 M, 5 mL) was added to low, mid, and high QC concentrations. The concentration after the addition of NaOH solution is equal to the original QCs. This solution was allowed to stand for 3 h at 60˚C in a closed container, then cooled to room temperature and neutralized with 5 M HCl to a

pH value of 7. Calibration curves for the three drugs were injected simultaneously with QCs. Considerable losses were observed. The relative recovery for: (HCT) 37%, (Am) 62%, and (Val) 49%.

3.8. Acidic Conditions

Hydrochloric acid (HCl) (1.0 M, 5 mL) was used to perform this test. A similar test procedure was applied. Better recovery was observed. The relative recovery for: (HCT) 77%, (Am) 82%, and (Val) 72%.

4. Conclusion

A rapid and simple HPLC/UV and HPLC/Fl methods have been described for the simultaneous analysis of hydrochlorothiazide (HCT), Amlodipine (Am), and Valsartan (Val) drugs in tablets. An XE-60-S-valine-S-phenylethylamide (Chrompack) column, 25 m × 0.25 mm I.D. and 0.12 μm film thickness, was employed. The chromatographic elution step is undertaken in a short time with high resolution. The calibration curves were linear over the concentration range of 0.040 - 1.50 μg/mL for the three drugs. The method is accurate (bias < 2.60%) and reproducible (SD < 7%), with a quantization limit of 0.010 μg/mL. Analytical recoveries were >96%. This assay is suitable for biomedical applications.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

References

[1] Hajilo, S. and Sohrabi, M.R. (2025) Spectrophotometric Method Based on Continuous Wavelet Transform and Ratio Substraction Methods for the Rapid Simultaneous Quantification of Valsartan, Amlodipine, and Hydrochlorothiazide in Cardiovascular Tablet Formulation. *Spectrochimica Acta Part A*: *Molecular and Biomolecular Spectroscopy*, **330**, Article ID: 125715. https://doi.org/10.1016/j.saa.2025.125715

[2] Shaikh, J.S.A., Raut, S., Abdul, A. and Pathan, M.A.A.K. (2020) High Performance Liquid Chromatographic Assay of Amlodipine, Valsartan and Hydrochlorothiazide Simultaneously and Its Application to Pharmaceuticals, Urine and Plasma Analysis. *Journal of Chromatography B*, **1155**, Article ID: 122295. https://doi.org/10.1016/j.jchromb.2020.122295

[3] Shah, J.V., Parekh, J.M., Shah, P.A., Shah, P.V., Sanyal, M. and Shrivastav, P.S. (2017) Application of an LC-MS/MS Method for the Analysis of Amlodipine, Valsartan and Hydrochlorothiazide in Polypill for a Bioequivalence Study. *Journal of Pharmaceutical Analysis*, **7**, 309-316. https://doi.org/10.1016/j.jpha.2017.06.001

[4] Gadepalli, S.G., Deme, P., Kuncha, M. and Sistla, R. (2014) Simultaneous Determination of Amlodipine, Valsartan and Hydrochlorothiazide by LC-ESI-MS/MS and Its Application to Pharmacokinetics in Rats. *Journal of Pharmaceutical Analysis*, **4**, 399-406. https://doi.org/10.1016/j.jpha.2013.12.003

[5] Darwish, H.W., Hassan, S.A., Salem, M.Y. and El-Zeany, B.A. (2014) Different Approaches in Partial Least Squares and Artificial Neural Network Models Applied for the Analysis of a Ternary Mixture of Amlodipine, Valsartan and Hydrochlorothiazide. *Spectrochimica Acta Part A*: *Molecular and Biomolecular Spectroscopy*, **122**,

744-750. https://doi.org/10.1016/j.saa.2013.11.045

[6] Shaalan, R.A., Belal, T.S., El Yazbi, F.A. and Elonsy, S.M. (2017) Validated Stability-Indicating HPLC-DAD Method of Analysis for the Antihypertensive Triple Mixture of Amlodipine Besylate, Valsartan and Hydrochlorothiazide in Their Tablets. *Arabian Journal of Chemistry*, **10**, S1381-S1394. https://doi.org/10.1016/j.arabjc.2013.04.012

[7] Lotfy, H.M., Hegazy, M.A., Mowaka, S. and Mohamed, E.H. (2015) Novel Spectrophotometric Methods for Simultaneous Determination of Amlodipine, Valsartan and Hydrochlorothiazide in Their Ternary Mixture. *Spectrochimica Acta Part A*: *Molecular and Biomolecular Spectroscopy*, **140**, 495-508. https://doi.org/10.1016/j.saa.2014.12.096

[8] van der Nagel, B.C.H., Versmissen, J., Bahmany, S., van Gelder, T. and Koch, B.C.P. (2017) High-Throughput Quantification of 8 Antihypertensive Drugs and Active Metabolites in Human Plasma Using UPLC-MS/MS. *Journal of Chromatography B*, **1060**, 367-373. https://doi.org/10.1016/j.jchromb.2017.06.029

[9] Ahmed, A.R., Galal, S.M., Korany, M.A., Elsheikh, M.A., Bedair, A.F. and Ragab, M.A.A. (2024) Bioanalytical HPLC Method with Fluorescence Detector for Determination of Entresto™ When Co-Administered with Ibuprofen and Fexofenadine: A Pharmacokinetic Study. *RSC Advances*, **14**, 19197-19205. https://doi.org/10.1039/d4ra02163k