Letters in Applied NanoBioScience
Open-Access Journal (ISSN: 2284-6808)

Article
Volume 12, Issue 4, 2023, 162
https://doi.org/10.33263/LIANBS124.162

The New Use of High-Performance Liquid Chromatography Technology to Follow the Stages of Synthesis of Sulfonamides and the Calculation of their Yield Directly

Souad Akili [1], Djamila ben Hadda [2], Yaser Bitar [1], Mustapha Fawaz Chehna [1,*]

[1] Department of Pharmaceutical Chemistry and Drug Control, Faculty of Pharmacy, University of Aleppo, Aleppo, Syria
[2] Department of Pharmaceutical Chemistry and Drug Control, Faculty of Pharmacy, Ebla Private University, Aleppo, Syria
* Correspondence: e mf.chehna@gmail.com (M.F.C.);

Scopus Author ID 35798009400
Received: 4.01.2022; Accepted: 29.04.2022; Published: 21.11.2022

Abstract: High-performance liquid chromatography has been used as a modern analytical method in tracking the reaction of compound 1a synthesis from sulfamethoxazole rather than classical methods such as thin-layer chromatography (TLC). The analytical conditions for separating compound 1a from the initial substances involved in the reaction have been determined. The method's validity was studied per the conditions recommended by the International Conference on Harmonization (ICH). The reaction yield is then directly calculated using the resulting high-performance liquid chromatography scheme. The analytical method was developed using the following chromatographic conditions: column C18 and the mobile phase composed of distilled water: acetonitrile: methanol 60:35:5 v/v, and apparent pH of 2.5 was adjusted with phosphorous acid, and the mobile phase flow rate was 1 ml/min, UV detection wavelength 278 nm, temperature 30°C. The retention times of sulfamethoxazole and compound 1a were (5, 13.5) minutes, respectively. The developed method was validated for accuracy, precision, selectivity, and robustness, the developed method was given acceptable linearity (R^2>0.999) within the range (0.005-0.025) mg/ml, and the result of the precision study for this method was (RSD%<2%), and the mean value of recovery was (98.47-101.52)% for sulfamethoxazole and (98.53-101.45)% for compound 1a and therefore the developed method was precision and accurate. The validated method was used to calculate the yield of compound 1a synthesis, in which the yield ratio of sulfamethoxazole and compound 1a of the resulting peaks was 17.565 and 81.325 for sulfamethoxazole and compound 1a, respectively. And also was used in monitoring of compound 1b synthesis reaction, which the yield ratio was after one hour of synthesis reaction 65.927 and 32.363 for sulfamethoxazole and compound 1b, respectively.

Keywords: high-performance liquid chromatography; HPLC; sulfonamides; sulfamethoxazole; sulfonamide synthesis; synthesis yield.

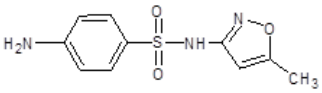
1. Introduction

The production of sulfonamides in 1935 marked an important turning point in antibiotics. The age of magic drugs began to emerge; as penicillin was discovered and followed by other antibiotics, sulfonamides became less used. Sulfonamides began to attract considerable attention after mobile towards synergistic activity, especially when combined with trimethoprim [1].

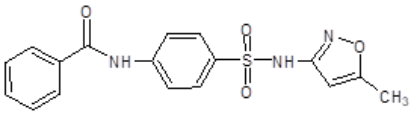
The discovery of antimicrobial *Prontosil rubrum* in the early 1930s marked the beginning of the development of chemotherapy. In 1932, the German chemist Gerhard Domage discovered a prontosil that controlled streptococcal infections in mice and later received the Nobel Prize in 1939 in physiology and medicine. Prontosil is an azo pigment associated with the general structure of sulfonamides [2,3]. Sulfonamide and amide derivatives form an important class of drugs with various biological applications [4].

More than 30 drugs containing this group are currently in clinical use. Sulfonamides are widely used as antimicrobials, antiprotozoal and antifungal, anti-inflammatory, antihypertensive, anti-malarial, anti-depressant and anti-thyroid, and diuretic [4–9]. It is also effective for treating urinary, intestinal, and ophthalmic infections and ulcerative colitis [10]. Recently, sulfonamides have been used as an anti-cancer and anti-virus inhibitor of HIV protease [11–15].

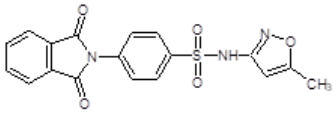
Previous articles by our informant on the synthesis and design of some new sulfonamide derivatives have been published as anti-cancer agents. The molecular docking study of compounds designed against the receptor of EGFR and the receptor of T790M/L858R epidermal growth factor (TMLR) was conducted to identify new candidate drugs for cancer treatment. Some designed sulfonamide derivatives have been synthesized using simple methods and require one synthesis stage to obtain the required compound. Microwave heating has also been used to synthesize compounds in a shorter time, at higher temperatures, and yield higher than conventional synthesis [16,17]. Figure 1 shows the structure of sulfamethoxazole, 1a, and 1b compound.



Sulfamethoxazole (4-amino-*N*-(5-methyl-1,2-oxazol-3-yl)benzenesulfonamide)



1a (N-4-[(5-methyl-1,2-oxazol-3-yl)sulfamoyl]phenyl}benzamide)



1b (4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-*N*-(5-methyl-1,2-oxazol-3-yl)benzensulfonamide)

Figure 1. Structure of sulfamethoxazole and compounds (1a, 1b).

Several analytical methods have been developed to identify and quantify sulfonamides as raw materials and pharmaceuticals, including high-performance liquid chromatography (HPLC), liquid chromatography associated with a mass spectrometer (LC-MS), gas chromatography (GC), and capillary electrophoresis (CE). Several studies identify and calibrate sulfonamides using HPLC-UV [18–22], and several conditions are used in various articles to separate the sulfamethoxazole compound from trimethoprim and other compounds [23–26]. But they have rarely been used to monitor certain drugs' synthesis reactions and separate them from raw materials. A simple, reliable, inexpensive, quick, and less environmentally dangerous routine method is still needed in pharmaceutical synthesis reactions to determine the benefits of these interactions. The classical methods of thin-layer

chromatography (TLC) were often used to follow the stages of synthesizing such compounds and then separate them from the reaction medium and re-crystallizing them to calculate the reaction yield. In our study, we used high-performance liquid chromatography technology with the UV detector HPLC-UV to follow the synthesis reaction and then directly calculate the yield for their accuracy in separating compounds and determining their combinations without the need for classical methods.

2. Materials and Methods

2.1. Materials.

Standard sulfamethoxazole with 99% purity and compounds (1a, 1b) were used. To prepare the mobile phase, some solvents have been used, including acetonitrile prepared for use in HPLC liquid chromatography with a purity of 99.9% (Lab-Scan LTD, Ireland), methanol is prepared for use in HPLC liquid chromatography with 99.7% purity (Fischer, Germany), also, 85% phosphoric acid (Scharlau, Spain), 37% HCl (Panreac Quimica Saua, Spain), and 99% NaOH (Merck, Germany).

2.2. Instrumentation.

The Sensitive Balance ±0.1 (Sartorius, model 2215, Germany), a device measuring the acidity of the medium (pH) ModelGLP21 (Crison, Spain), water bathroom powered by Power-Sonic 405 (Hwashin Technology, Korea), High-performance liquid chromatography device, EZCHrom Elite, Version 3.2.1 (Agilent, Germany), is powered by a pump with a detector (UV-RID), a 250 mm long, 4.6 mm long C18 separation column packed with 5 mm diameter grains (Thermo Fisher Scientific, America).

2.3. Preparing standard solutions.

A standard solution for sulfamethoxazole was prepared with a concentration (0.2 mg/ml), weighing 20 mg of sulfamethoxazole and dissolved with 100 ml of methanol. The standard work solutions were prepared by taking increasing volumes from the parent standard solution (0.2 mg/ml) to several 10 ml standard volumetric flasks and completing the volume by the mobile phase to get several concentrations between (0.005-0.025) mg/ml, and compound 1a was prepared in the same way as sulfamethoxazole.

2.4. Preparation of degradation solutions.

Ruining by light: under the previously adopted preparation method, the standard work solution of sulfamethoxazole and compound 1a was prepared with a concentration (0.2mg/ml), then taken to 0.5 ml of the standard solution, transferred to a 10 ml volumetric flask and extended the methanol up to the signal to obtain a 0.01mg/mL concentration solution and then save the solution in a place that's exposed to sunlight for a week at room temperature.

Ruined by acid hydrolysis: the standard work solution with a concentration (of 0.2 mg/ml) was prepared under the previously adopted preparation method, then was taken 0.5 ml of the standard solution and transferred to a 10 ml volumetric flask containing 0.5 ml HCl (1 N) and extended with methanol until the signal to obtain a 0.01 mg/ml concentration solution and then save the solution at room temperature for a week.

Ruined by the alkaline hydrolysis: the standard work solution with a concentration (of 0.2 mg/ml) was prepared under the previously adopted preparation method, then was taken 0.5 ml of the standard solution and transferred to a 10 ml volumetric flask containing NaOH 0.5 ml (1N) and extended with methanol until the signal to obtain a 0.01 mg/ml concentration solution and then save the solution at room temperature for a week.

2.5. Method development and optimization of chromatographic conditions.

2.5.1. Selection of detection wavelength.

The detection wavelengths were λ max 278 nm for sulfamethoxazole and compound 1a.

2.5.2. Column selection.

C18- reversed-phase column, 250 x 4.6 mm 5-µm, was utilized.

2.5.3. Mobile phase preparation.

The mobile phase consisting of 60 mL distilled water, 35 mL acetonitrile, and 5 mL methanol, and apparent pH of 2.5 was adjusted with phosphoric acid.

2.5.4. Analytical method validation.

Method validation was performed according to International Conference on Harmonization (ICH) recommended test conditions [27].

3. Results and Discussion

Most previous studies have relied on thin-layer chromatography to track organic or pharmacological synthesis reactions. In addition to being the key to determining the end of the reaction, the methods for calculating the yield of such interactions were based on the separation of the resulting compounds and their purification of them by recrystallization. In our study, we looked at finding a simple, quick, and even less expensive method of using solutions that would be useful in tracking the reaction of sulfonamides and calculating the yield of the reaction. We used high-performance liquid chromatography technology in the study because it corresponds to the range required to follow drug synthesis reactions and calculate yields based on peak area in HPLC chromatograms. Therefore, it was necessary to determine the optimal conditions for separating compound 1a from sulfamethoxazole and verify the method's validity.

3.1. HPLC analysis.

The chromatographic conditions comprised a C18 reversed-phase column, 250 x 4.6 mm 5-µm, with a mobile phase consisting of 60 mL distilled water, 35 mL acetonitrile, and 5 mL methanol, and apparent pH of 2.5 was adjusted with phosphoric acid. The flow rate was 1 ml/min, and the detection wavelength was UV 278 nm, using a temperature of 30°C. The standard solutions for sulfamethoxazole and compound 1a were injected into the HPLC using chromatographic conditions. The retention time was 5 minutes for sulfamethoxazole and 13.5 minutes for compound 1a (Figure 2). The tailing factor was 1.33 for sulfamethoxazole and 1.16 for compound 1a.

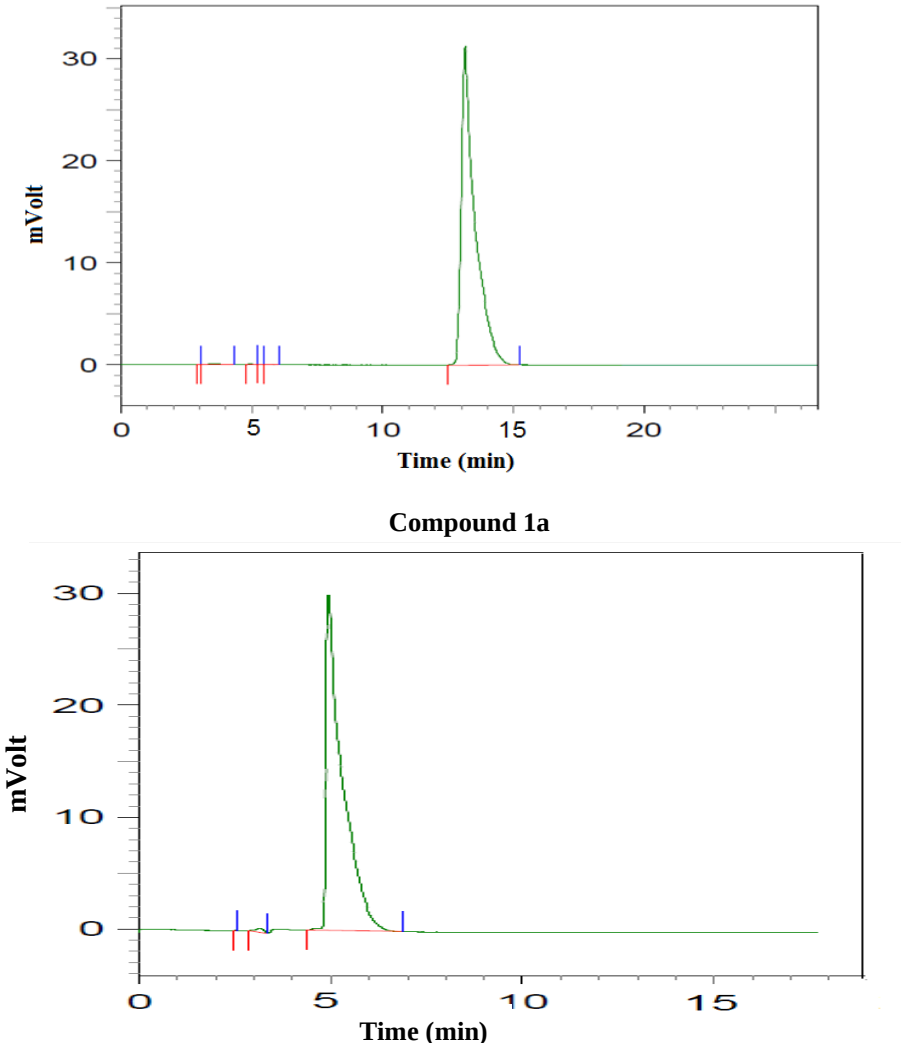


Figure 2. Chromatogram of sulfamethoxazole and compound 1a (0.01 mg/ml).

3.2. Validation of developed analytical method:

3.2.1. Linearity.

The linearity is the analytical method's ability to produce results directly proportional to the concentration of the analyzed material in the sample within the proposed range. The Linearity study was carried out on a series of standard solutions consisting of five concentrations within the range (0.005-0.025) mg/ml for sulfamethoxazole and compound 1a. The correlation coefficient was 0.99995 for sulfamethoxazole and compound 1a, indicating that the linear is good. Each sample was injected 3 replicates, the mean areas of the three peaks were calculated for each recorded concentration, and the relationship between the concentration (mg/ml) of the compound 1a solution and the sulfamethoxazole and the peak was drawn (Figure 3). The relationship between concentration and peak area has been shown to be linear within the range (0.005-0.025) mg/ml.

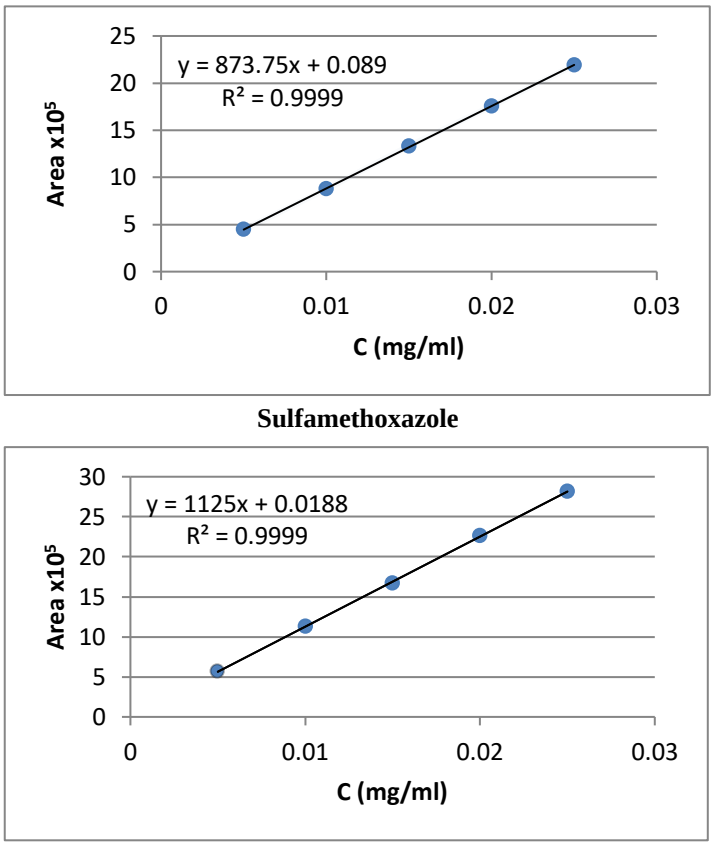


Figure 3. Calibration curve for sulfamethoxazole and compound 1a.

The values of the area in the resulting linear equation have been compensated, and the actual concentrations of the sulfamethoxazole and compound 1a have been calculated, as shown in Table 1:

Table 1. Linearity data for sulfamethoxazole and compound 1a.

	Sulfamethoxazole		Compound 1a	
Theoretical Conc. (mg /ml)	**Actual Conc. (mg/ml)±SD**	**RSD%**	**Actual Conc. (mg/ml)±SD**	**RSD%**
0.005	0.004991±0.044021	0.989248	0.0051±0.012298	0.215654
0.01	0.009919±0.014907	0.170245	0.01002±0.073782	0.653476
0.015	0.015146±0.045753	0.343432	0.014831±0.066122	0.395849
0.02	0.019986±0.124009	0.706541	0.020071±0.038286	0.169414
0.025	0.024958±0.090118	0.411576	0.025027±0.020086	0.071292

3.2.2. Accuracy.

The accuracy of the developed analytical method expresses the closeness of agreement between the measured practical and theoretical results, which are studied within the range of the analytical method. Recovery studies were performed for three replicates of three concentrations within the linearity range. The recovery results for sulfamethoxazole were (98.47-101.52)% and (98.53-101.45)% for compound 1a, Table 2,3, within the acceptable accuracy range of (98-102)%. This indicates that the developed method is accurate and applicable to the determination of sulfamethoxazole and compound 1a.

Table 2. Accuracy data for sulfamethoxazole.

Theoretical Conc. (mg /ml)	Actual Conc. (mg/ml)±SD	RSD%	Recovery%
0.005	0.005076±0.008528	0.19317	101.5275
0.01	0.009847±0.038237	0.44673	98.47049
0.015	0.015076±0.07589	0.57922	100.5088

Table 3. Accuracy data for compound 1a.

Theoretical Conc. (mg /ml)	Actual Conc. (mg/ml)±SD	RSD%	Recovery%
0.005	0.004927±0.035924	0.58761	98.53621
0.01	0.010146±0.069845	0.59815	101.4569
0.015	0.014926±0.105905	0.63139	99.50984

3.2.3. Precision.

The precision of the developed analytical method expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision was studied at two levels: repeatability and intermediate precision. Repeatability precision was established by injecting five replicate injections of standard solution (0.01mg/ml) for sulfamethoxazole and compound 1a; the results are shown in Table 4.

Table 4. Repeatability data for sulfamethoxazole and compound 1a.

Sample number	Sulfamethoxazole	Compound 1a
1	8.79007	11.43335
2	8.78634	11.49743
3	8.73772	11.33374
4	8.7165	11.45263
5	8.75685	11.33551
Mean±SD	8.757496±0.028161	11.41053±0.065375
RSD%	0.321565	0.572936

The Intermediate precision of the developed analytical method has also been studied by preparing three concentrations within the linear range of sulfamethoxazole and compound 1a (0.005-0.01-0.015) mg/ml. Each sample has been injected with three replicates. The standard deviation and relative standard deviation RSD% were calculated for each concentration and were less than 1%, as shown in Table 5.

Table 5. Intermediate precision data for sulfamethoxazole.

	Intra-day		Inter-day	
Theoretical Conc. (mg /ml)	**Actual Conc. (mg/ml)±SD**	**RSD%**	**Actual Conc. (mg/ml)±SD**	**RSD%**
0.005	0.005096±0.008993	0.203983	0.005093±0.030691	0.692918
0.01	0.009807±0.051026	0.592122	0.009814±0.042249	0.489125
0.015	0.015096±0.04904	0.36755	0.015093±0.04904	0.36755

Table 6. Intermediate precision data for compound 1a.

	Intra-day		Inter-day	
Theoretical Conc. (mg /ml)	**Actual Conc. (mg/ml)±SD**	**RSD%**	**Actual Conc. (mg/ml)±SD**	**RSD%**
0.005	0.004974±0.04336	0.738046	0.004968±0.088078	1.51971
0.01	0.010052±0.055633	0.489124	0.010063±0.051391	0.451959
0.015	0.014974±0.053988	0.323209	0.014968±0.090923	0.5432

The analytical procedure was repeated the following day, the preparation and injection of the updated samples from the three concentrations of both sulfamethoxazole and compound 1a, and the standard deviation and the relative percentage deviation of RSD% were calculated for the results of the two days of each concentration. The RSD% was calculated for the results of the two days (6 iterations) per concentration as it was less than 2%, as shown in Table 6.

3.2.4. Sensitivity.

Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated to evaluate the sensitivity of the developed analytical method. The calculated LOD and LOQ for sulfamethoxazole were 0.000284 and 0.000862 mg/ml, respectively. The calculated LOD and LOQ for compound 1a were 0.000319 and 0.000968 mg/ml, respectively.

3.2.5. Robustness.

To verify the robustness of the developed analytical method, minor changes were made to the chromatographic conditions applied to the studied compounds. To prove the robustness of the developed analytical method, standard solutions of the sulfamethoxazole and compound 1a were prepared and injected into three replicates, each time changing one of the chromatographic conditions. Changes were made to the mixing ratio of the mobile phase, the column's temperature, the mobile phase's flow rate, and the detection wavelength, depending on the relative percentage deviation of the RSD%. The results showed good robustness for the developed method since the RSD value was less than 2%, as shown in
Table 7.

Table 7. Robustness data for sulfamethoxazole and compound 1a.

		Sulfamethoxazole	**Compound 1a**
Factor	**Range of change**	**RSD%**	**RSD%**
Column Temperature (°C)	25	0.794145	0.335891
	30	0.277736	0.05974
	35	0.507288	0.632383
Flow Rate (ml/min)	0.9	0.367911	0.133903
	1	0.277736	0.05974
	1.1	0.651688	0.765019
Wavelength (nm)	277	0.659225	0.058364
	278	0.277736	0.05974
	279	0.963388	0.70995
Mobile Phase Composition (water:acetonitrile:methanol)	(58:37:5)	0.790462	0.60545
	(60:35:5)	0.277736	0.05974
	(62:33:5)	0.158395	0.075374

3.2.6. Specificity.

The ability of the developed analytical method to identify the studied compound by the presence of other substances that are expected to exist in the sample as degraded compounds from the studied compound. A standard solution of sulfamethoxazole and compound 1a with a concentration of 0.01mg/ml was prepared, and then it was kept in a place that's exposed to sunlight for a week at room temperature. After the expiration of the period, it was injected into the HPLC device, and recorded the resulting chromatogram. It was observed that there was an additional peak separate from the peak of the active compound by comparing it with the chromatogram corresponding to the sulfamethoxazole and compound 1a. The resolution factor was calculated between the active compound and neighboring peaks. The resolution factor

values were 2.5 and 17.77 for sulfamethoxazole and compound 1a, respectively, as shown in Figure 4.

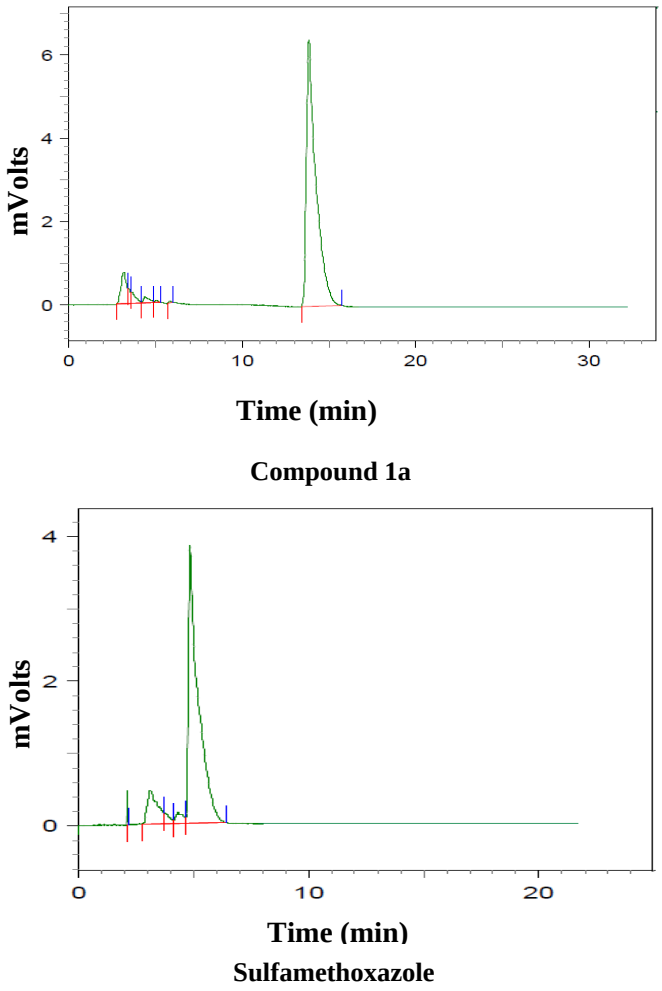


Figure 4. Chromatogram of the degradation solution by the sunlight of sulfamethoxazole and compound 1a.

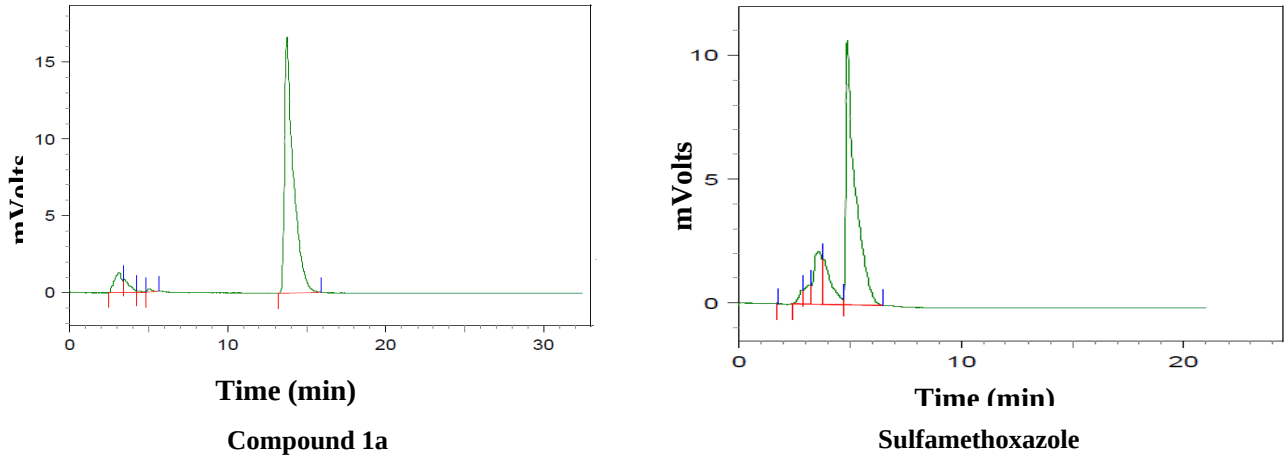


Figure 5. Chromatogram of the degradation solution by acid hydrolysis of sulfamethoxazole and compound 1a.

We performed forced destruction by acid hydrolysis of the standard solution of sulfamethoxazole and the compound 1a with a concentration of 0.01mg/ml using HCl (1N); the solution was conserved at room temperature for a week; after that, the sample was injected into the HPLC, and the resulting chromatogram was recorded, and by comparison with the

chromatogram approved for the sulfamethoxazole and compound 1a. We observed an additional peak in this chromatogram separate from the peak of the active compound. We observed an additional peak in this chromatogram separate from the peak of the active compound. The resolution factor was calculated between the active compound peak and the neighboring peak. The resolution factor values were 1.5 and 12.1 for sulfamethoxazole and compound 1a, respectively, as shown in Figure 5.

We also performed forced destruction by alkaline hydrolysis of the standard solution of sulfamethoxazole and the compound 1a with a concentration of 0.01mg/ml using NaOH (1N); the solution was conserved at room temperature for a week; then, the sample was injected into the HPLC, and the resulting chromatogram was recorded, In comparison with the chromatogram corresponding to the sulfamethoxazole and compound 1a, an additional peak is found separate from the main peak. The resolution factor between the active compound peak and the neighboring peak was calculated, and it was 1.81 and 11.8 for sulfamethoxazole and compound 1a, respectively, as shown in Figure 6.

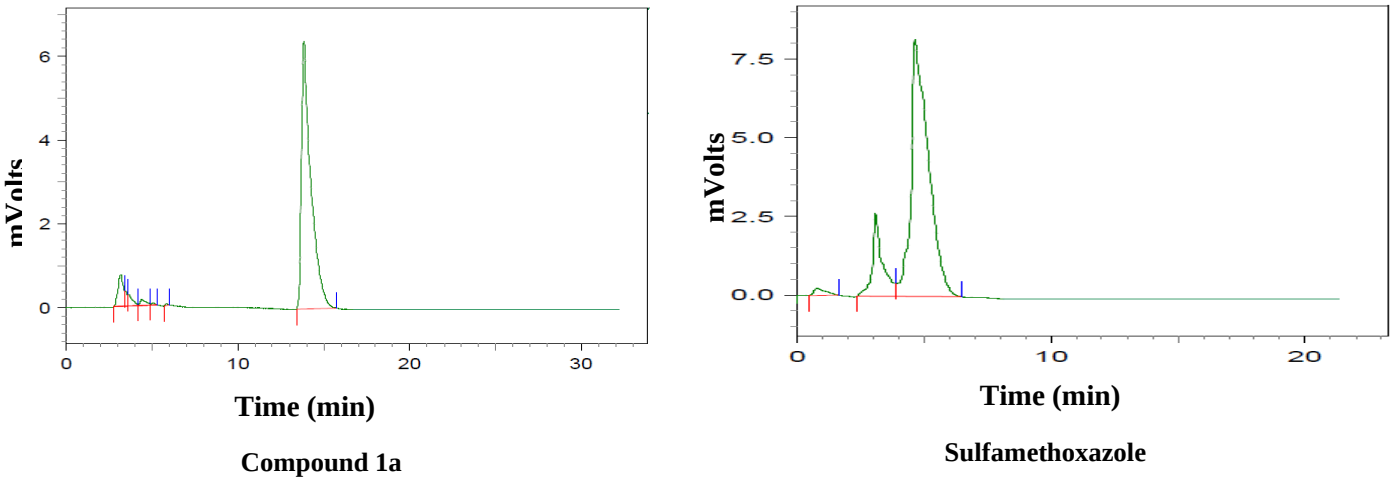


Figure 6. Chromatogram of the degradation solution by alkaline hydrolysis of sulfamethoxazole and compound 1a.

3.3. Separation and identification of a mixture containing sulfamethoxazole and compound 1a.

Previously developed chromatographic method conditions have been applied in separating and identifying sulfamethoxazole and compound 1a in a mixture containing a concentration of 0.01mg/ml of both substances so that the combination is similar to the synthesis reaction. The solution containing the material was injected into the HPLC device and was repeated three times in a row to increase accuracy, and we got Figure 7. Calculated the consonant peaks' corresponding area for sulfamethoxazole and compound 1a. Then the resulting area values were then offset in the linear equation of the calibration curves for both sulfamethoxazole and compound 1a, and the practical combinations for sulfamethoxazole and compound 1a were calculated. The standard deviation and the relative percentage deviation of RSD% were calculated as shown in Table 8.

Table 8. Results of quantification of the sulfamethoxazole mixture and compound 1a

Studied Compound	Theoretical Conc. (mg /ml)	Actual Conc. (mg/ml)	Recovery% ± SD	RSD%
Sulfamethoxazole	0.01	0.010124	101.2423±0.012525	0.140175
Compound 1a	0.01	0.009917	99.16599±0.055186	0.493835

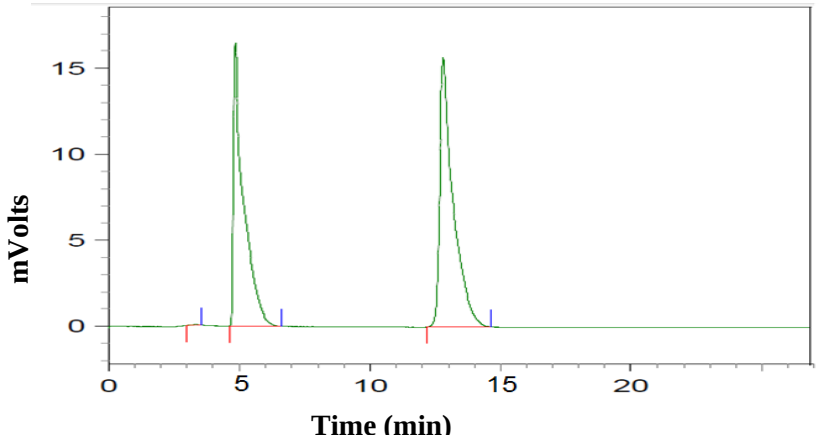


Figure 7. Chromatogram for combination sulfamethoxazole and compound 1a.

3.4. Applications of the developed analytical method to calculate the yield of the pharmaceutical synthesis reaction.

3.4.1. Calculation of the yield of compound 1a synthesis via HPLC.

The conditions of the developed chromatographic method were applied to the result of the synthesis reaction of compound 1a from the sulfamethoxazole. The compound was synthesized in the classical manner mentioned in our previous article [17]. After the reaction ended, the resulting compound was filtered and dried well after the solvents were vaporized. A solution of the synthesis product equivalent to 0.01 mg/mL was prepared by extending it with the mobile phase used in our method. The solution was injected into the HPLC device, Figure 8, and the approved peak area averages for sulfamethoxazole and compound 1a have been calculated to offset the resulting area values in the approved linear equations for standard curves of both sulfamethoxazole and compound 1a. The practical concentration was calculated, and the yield ratio of sulfamethoxazole and compound 1a of the resulting peaks was 17.565 and 81.325 for sulfamethoxazole and compound 1a, respectively. Table 9 compares this return with the classical method referred to in reference [17], which was 84%. It turns out to be very close, which proves the validity and accuracy of this method in addition to its speed.

Table 9. Results of synthesis yield of compound 1a using HPLC.

Studied Compound	Area	Actual Conc. (mg/ml)	Yield%
Sulfamethoxazole	1.50313	0.001618	17.565
compound 1a	7.12301	0.006315	81.325

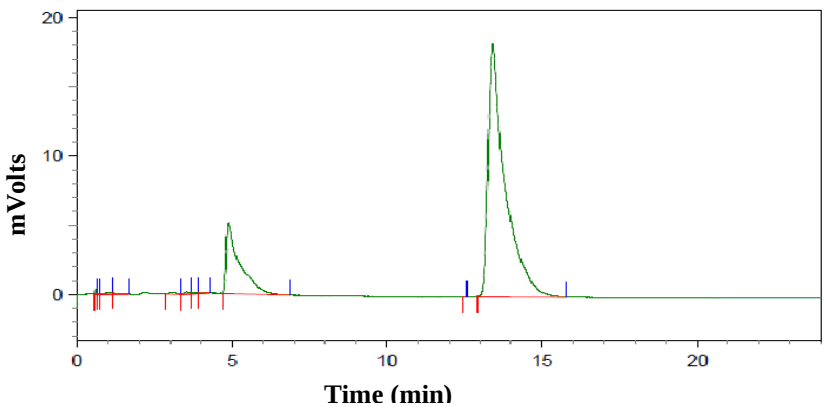


Figure 8. Chromatogram of the end product of compound 1a synthesis reaction.

3.4.2. Monitoring of compound 1b synthesis reaction via HPLC.

The conditions of the developed chromatographic method were applied to observe the reaction of compound 1b synthesis, where a sample was taken during the reaction after one hour to ensure that the formation of compound 1b began. A concentration solution of 0.01 mg/mL was prepared, and the solution was injected into the HPLC device, and this process was repeated three times in a row, and we got Figure 9.

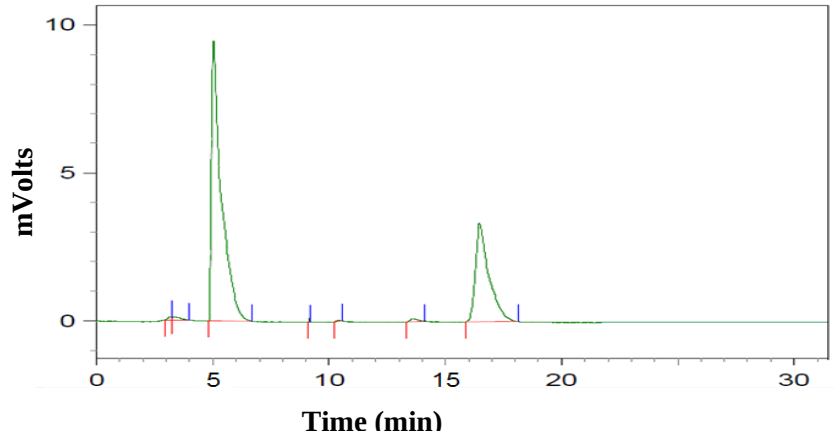


Figure 9. Chromatogram of the injector solution taken during the compound 1b synthesis reaction.

When the developed chromatographic conditions on the compound 1b synthesis reaction were applied, a top was obtained for the sulfamethoxazole with 5 minutes and the compound 1b with 16.4 minutes. The percentage yield of the synthesis reaction was obtained from the resulting peaks, which were 65.927 and 32.363 for sulfamethoxazole and compound 1b, respectively, Table 10.

Table 10. Results yield the injector solution taken during the compound 1b synthesis reaction of compound 1b using HPLC.

Studied Compound	Area	Yield%
sulfamethoxazole	27.0237	65.927
compound 1b	13.2657	32.363

4. Conclusions

A new analysis method characterized by linear, precision, accuracy, robustness, and specificity have been developed within the studied range of both sulfamethoxazole and compound 1a., The developed method was used to calculate the reaction yield of compound 1a and compare that with the classic method; it was close to the results, but our new method was characterized by speed and accuracy. The reaction of compound 1b synthesis was monitored. So the high-performance liquid chromatography method is more rapid and accurate in predicting the stages of evolution of the synthesis reaction and calculating the reaction yield.

Funding

This research received no external funding.

Acknowledgments

This research has no acknowledgment.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Schneider, Y.K. Bacterial Natural Product Drug Discovery for New Antibiotics: Strategies for Tackling the Problem of Antibiotic Resistance by Efficient Bioprospecting. *Antibiotics* **2021**, *10*, 1–15, https://doi.org/10.3390/antibiotics10070842.
2. Tacic, A.; Nikolic, V.; Nikolic, L.; Savic, I. Antimicrobial Sulfonamide Drugs. *Adv. Technol.* **2017**, *6*, 58–71, https://doi.org/10.5937/savteh1701058t.
3. Christensen, S.B. Drugs That Changed Society: History and Current Status of the Early Antibiotics: Salvarsan, Sulfonamides, and β-Lactams. *Molecules* **2021**, *26*, 1–32, https://doi.org/10.3390/molecules26196057.
4. Saeedi, M.; Goli, F.; Mahdavi, M.; Dehghan, G.; Faramarzi, M.A.; Foroumadi, A.; Shafiee, A. Synthesis and Biological Investigation of Some Novel Sulfonamide and Amide Derivatives Containing Coumarin Moieties. *Iran. J. Pharm. Res.* **2014**, *13*, 881–892, https://doi.org/10.22037/ijpr.2014.1528.
5. Kaur, L.; Arora, V.; Arora, P.; Singh, M.P. A Review: Biological Significance of Sulfonamides. *World Journal Of Pharmacy And Pharmaceutical Sciences* **2018**, *7*, 413–422, https://doi.org/10.20959/wjpps20184-11234.
6. Chohan, Z.H. Metal-Based Antibacterial and Antifungal Sulfonamides: Synthesis, Characterization, and Biological Properties. *Transit. Met. Chem.* **2009**, *34*, 153–161, https://doi.org/10.1007/s11243-008-9171-y.
7. Spisz, P.; Chylewska, A.; Krolicka, A.; Ramotowska, S.; Dabrowska, A.; Makowski, M. Stimulation of Sulfonamides Antibacterial Drugs Activity as a Result of Complexation with Ru (III): Physicochemical and Biological Study. *Int. J. Mol. Sci.* **2021**, *22*, 1–25, https://doi.org/10.3390/ijms222413482.
8. Ovung, A.; Bhattacharyya, J. Sulfonamide Drugs: Structure, Antibacterial Property, Toxicity, and Biophysical Interactions. *Biophys. Rev.* **2021**, *13*, 259–272, https://doi.org/10.1007/s12551-021-00795-9.
9. Heiran, R.; Jarrahpour, A.; Riazimontazer, E.; Troudi, A.; Giorgio, C. Di; Brunel, J.M.; Turos, E.; Heiran, R.; Jarrahpour, A.; Riazimontazer, E.; Gholami, A.; Troudi, A. Novel Sulfonamide β-Lactam Hybrids Incorporating the Piperazine Moiety as Potential INOS Inhibitors with Promising Antibacterial Activity. *Chemistry Select* **2021**, *6*, 5313–5319, https://doi.org/10.1002/slct.202101194.
10. Rehman, H.; Qadir, A.; Ali, Z.; Nazir, S.; Zahra, A.; Shahzady, T.G. Synthesis and Characterization of Novel Sulfonamides Derivatives and Their Antimicrobial, Antioxidant and Cytotoxicity Evaluation. *Bull. Chem. Soc. Ethiop.* **2017**, *31*, 491–498, https://doi.org/10.4314/bcse.v31i3.13.
11. Rathish, I.G.; Javed, K.; Ahmad, S.; Bano, S.; Alam, M.S.; Akhter, M.; Pillai, K.K.; Ovais, S.; Samim, M. Synthesis and Evaluation of Anticancer Activity of Some Novel 6-Aryl-2-(p-Sulfamylphenyl)-Pyridazin-3(2H)-Ones. *Eur. J. Med. Chem.* **2012**, *49*, 304–309, http://dx.doi.org/10.1016/j.ejmech.2012.01.026.
12. Gornowicz, A.; Szymanowska, A.; Mojzych, M.; Czarnomysy, R.; Bielawski, K.; Bielawska, A. The Anticancer Action of a Novel 1,2,4-Triazine Sulfonamide Derivative in Colon Cancer Cells. *Molecules* **2021**, *26*, 1–24, http://dx.doi.org/10.3390/molecules26072045.
13. Fatahala, S.S.; Sayed, A.I.; Mahgoub, S.; Taha, H.; El-Sayed, M.I.K.; El-Shehry, M.F.; Awad, S.M.; El-Hameed, R.H.A. Synthesis of Novel 2-Thiouracil-5-Sulfonamide Derivatives as Potent Inducers of Cell Cycle Arrest and Cdk2a Inhibition Supported by Molecular Docking. *Int. J. Mol. Sci.* **2021**, *22*, 1–23, http://dx.doi.org/10.3390/ijms222111957.
14. Thushara, N.; Darshani, T.; Samarakoon, S.R.; Perera, I.C.; Fronczek, F.R.; Sameera, W.M.C.; Perera, T. Synthesis, Characterization and Biological Evaluation of Dipicolylamine Sulfonamide Derivatized Platinum Complexes as Potential Anti-cancer Agents. *RSC Adv.* **2021**, *11*, 17658–17668, http://dx.doi.org/10.1039/d1ra00842k.
15. Nemr, M.T.M.; AboulMagd, A.M.; Hassan, H.M.; Hamed, A.A.; Hamed, M.I.A.; Elsaadi, M.T. Design, Synthesis and Mechanistic Study of New Benzenesulfonamide Derivatives as Anticancer and Antimicrobial Agents via Carbonic Anhydrase IX Inhibition. *RSC Adv.* **2021**, *11*, 26241–26257, http://dx.doi.org/10.1039/d1ra05277b.
16. Akili, S.; Ben Hadda, D.; Bitar, Y.; Najjar, A.; Chehna, M.F. Computer-Aid Design of Novel Sulfonamide Derivatives as EGFR Kinase Inhibitors for Cancer Treatment. *Int. J. Org. Chem.* **2021**, *11*, 171–186, http://doi.org/10.4236/ijoc.2021.114012.
17. Akili, S.; Ben Hadda, D.; Bitar, Y.; Balash, A.; Fawaz Chehna, M. Design, Synthesis and Characterization of Novel Sulfonamides Derivatives as Anticancer Agent Targeting EGFR TK, and Development of New Methods of Synthesis by Microwave Irradiation. *Int. J. Org. Chem.* **2021**, *11*, 199–223, https://doi.org/10.4236/ijoc.2021.114014.
18. Patyra, E.; Przeniosło-Siwczynska, M.; Kwiatek, K. Determination of Sulfonamides in Feeds by High-Performance Liquid Chromatography after Fluorescamine Precolumn Derivatization. *Molecules* **2019**, *24*, 1–12, https://doi.org/10.3390/molecules24030452.

19. Kim, H.J.; Jeong, M.H.; Park, H.J.; Kim, W.C.; Kim, J.E. Development of an Immunoaffinity Chromatography and HPLC-UV Method for Determination of 16 Sulfonamides in Feed. *Food Chem.* **2016**, *196*, 1144–1149, http://doi.org/10.1016/j.foodchem.2015.10.014.
20. Patyra, E.; Kwiatek, K. Application of Micellar Mobile Phase for Quantification of Sulfonamides in Medicated Feeds by Hplc-Dad. *Molecules* **2021**, *26*, 1–12, http://doi.org/10.3390/molecules26133791.
21. Gaballah, M.S.; Li, X.; Zhang, Z.; Al-Anazi, A.; Sun, H.; Sobhi, M.; Philbert, M.; Ghorab, M.A.; Guo, J.; Dong, R. Determination of Tetracycline, Oxytetracycline, Sulfadiazine, Norfloxacin, and Enrofloxacin in Swine Manure Using a Coupled Method of On-Line Solid-Phase Extraction with the UHPLC–DAD. *Antibiotics* **2021**, *10*, 1397, http://doi.org/10.3390/antibiotics10111397.
22. Dowlatshah, S.; Santigosa, E.; Saraji, M.; Ramos, M. A Selective and Efficient Microfluidic Method-Based Liquid Phase Microextraction for the Determination of Sulfonamides in Urine Samples. *J. Chromatogr. A* **2021**, *1652*, 1–6, https://doi.org/10.1016/j.chroma.2021.462344.
23. Asadi, S.; Gharbani, P. Simultaneous Determination of Sulfamethoxazole and Phthalazine by HPLC and Multivariate Calibration Methods. *Iran. J. Chem. Chem. Eng.* **2013**, *32*, 1–8.
24. Irungu, B.N.; Koech, L.C.; Ondicho, J.M.; Keter, L.K. Quality Assessment of Selected Cotrimoxazole Suspension Brands Marketed in Nairobi County, Kenya. *PLoS One* **2021**, *16*, 1–13, http://doi.org/10.1371/journal.pone.0257625.
25. Barreca, S.; Forni, C.; Colzani, L.; Clerici, L.; Daverio, D.; Dellavedova, P. Study on the Stability of Antibiotics, Pesticides and Drugs in Water by Using a Straightforward Procedure Applying Hplc-Mass Spectrometric Determination for Analytical Purposes. *Separations* **2021**, *8*, 0–9, https://doi.org/10.3390/separations8100179.
26. Almeida, C.M.M. Overview of Sample Preparation and Chromatographic Methods to Analysis Pharmaceutical Active Compounds in Waters Matrices. *Separations* **2021**, *8*, 1–50, http://doi.org/10.3390/separations8020016.
27. Guy, R.C. Emea. ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology. *Eur. Med. Agency* **1995**, 1–15, https://doi.org/10.1016/B978-0-12-386454-3.00861-7.