Scientific Research Publishing

Open Journal of Optimization, 2023, 12, 11-24
https://www.scirp.org/journal/ojop
ISSN Online: 2325-7091
ISSN Print: 2325-7105

Optimization of a Method for the Simultaneous Determination of Phloroglucinol and Trimethylphloroglucinol by High Performance Liquid Chromatography in Routine Use in a Pharmaceutical Industry in Abidjan

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How to cite this paper: Kouamé, J.-K., Yéhé, M.D., Ngantchouko, C.B.N., Brou, A.G., Ablé, C.N., Kouadio, A.M.D., Vagny, B.G.D., De Paul Ovi, V., Amin, C.N. and Gbassi, G.K. (2023) Optimization of a Method for the Simultaneous Determination of Phloroglucinol and Trimethylphloroglucinol by High Performance Liquid Chromatography in Routine Use in a Pharmaceutical Industry in Abidjan. *Open Journal of Optimization*, **12**, 11-24.
https://doi.org/10.4236/ojop.2023.122002

Received: March 25, 2023
Accepted: June 3, 2023
Published: June 6, 2023

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Abstract

In order to provide the population with safe, effective and good quality medicines, the pharmaceutical industries, before releasing batches of their products into the pharmaceutical circuit, put in place internal dosage methods to control the quality of these products. The present study consisted in optimizing a method for the simultaneous determination of Phloroglucinol (PHG) and Trimethylphloroglucinol (TPH) by high performance liquid chromatography (HPLC) routinely used in a pharmaceutical industry located in a township in Abidjan (Ivory Coast). The basic chromatographic conditions were those routinely used for the determination of these two molecules: mobile phase: acetonitrile/water (60/40), stationary phase (C18 BDS Hypersil 250 mm * 4.6 mm - 5 µm), detection wavelength (265 nm), flow rate, injection volume and run time configured at the equipment level were respectively 1 mL/min, 10 µL and 8 min. The method of preparation of the analytes (PHG and TPHG) was also applied by the pharmaceutical industry. The application of these different parameters at the equipment level made it possible to determine a chromatogram which highlights three chromatographic peaks with respective retention times (RT) of 0.773 min (unidentified compound), 2.275 min (PHG) and 7.269 min for an analysis time of 8 min with a better resolu-

tion of the peaks and baseline. The progressive optimization of different parameters such as the stationary phase (C18 YMC 150 mm * 4.6 mm - 3 µm), the proportion of the mobile phase: acetonitrile/water (80/20), the flow rate impelled by the pump (0.8 mL/min) and the modification of the analyte preparation mode (same amount of PHG and TPHG in a 50 mL volumetric flask) resulted in a final chromatogram that highlighted two chromatographic peaks at the respective RT of 2.391 min (PHG) and 3.735 min (TPHG) at a run time of 6 min. The chromatographic conditions that led to the final chromatogram can be used routinely by the pharmaceutical industry for the determination of several PHG and TPHG drug matrices after prior validation of the determination method.

Keywords

PHG, TPHG, Pharmaceutical Industry, Chromatographic Profile, Chromatographic Peak

1. Introduction

A WHO study has shown that in developing countries, one tenth of the medicines on the market are substandard or even falsified [1] [2]. In response to this public health problem, many pharmaceutical industries and quality control laboratories have developed valid and reliable analytical methods to ensure that their pharmaceutical products on the market meet the assigned requirements [3] [4].

The combination of Phloroglucinol (PHG) and Trimethylphloroglucinol (TPHG) developed by many manufacturers in several pharmaceutical specialities is no exception to this plague [1] [2] [3] [5]. These molecules have an important place in the management of pain caused by muscle spasms, particularly in gynaecology [6] [7]. They are not only used in gynaecology, but also in the symptomatic treatment of pain related to functional disorders of the digestive tract [8], the biliary tract [9], in spasmodic manifestations and in acute pain of the urinary tract (renal colic) [10] [11]. According to a study conducted by Lecarpentier C in 2013 [12], PHG and TPHG represent 38.60% of the molecules prescribed during pregnancy and are also consumed in self-medication during pregnancy. These numerous pharmacological activities mentioned above make these molecules essential in the management of several pathologies in most developing countries and more particularly in Ivory Coast [13].

Given the importance that these molecules have in the therapeutic arsenal of the Ivorian population, it is important that the pharmaceutical industries based on the Ivorian territory make available to the population, safe, effective, quality and low-cost drugs by developing in-house reliable, reproducible analytical methods with reduced turnaround time. It is with the aim of accelerating the production of pharmaceutical products based on PHG and TPHG in all existing

presentations (tablets, injectables and suppositories) on the Ivorian pharmaceutical market with the advantage of reducing production costs for the industry and reducing stock-outs for patients that this study was initiated. To be achieved, various analytical methods are used by these industries such as: High Performance Liquid Chromatography (HPLC) [14], UV-visible Spectrophotometry [15], Gas Chromatography (GC) [16], mass spectrometry and capillary electrophoresis [17]. Among these techniques, HPLC/UV is used by several pharmaceutical industries to develop methods for the determination of their drug matrices as it has easy access and many qualitative and quantitative advantages over the other techniques listed above [18].

The aim of this study was to improve the quality of PHG and TPHG-based medicines released into the pharmaceutical circuit by a pharmaceutical industry in Abidjan Ivory Coast.

2. Materials and Method

2.1. Materials

2.1.1. Sampling

The reference substances Phloroglucinol Dihydrate (PHG) and Trimethylphloroglucinol (TPHG) were provided by the pharmaceutical industry for the study.

2.1.2. Reagents and Chemicals

Ultra pure water and HPLC grade Acetonitrile with batch number P1D666121E from the manufacturer CARLO ERBA were used to perform the study.

2.1.3. Apparatus

To carry out this study, the following equipment was used:

- A WATERS HPLC line (USA) composed of a pump (1525 binary HPLC pump), an automatic sampler (717 auto sampler), and a UV detector (2487) (**Figure 1**);
- A KERN (Germany) analytical balance for weighing;
- C18 BDS Hypersil (250 mm * 4.6 mm) 5 µm and C18 YMC (150 mm * 4.6 mm) 3 µm columns from the respective manufacturers THERMO SCIENTIFIC (USA) and YMC (Japan).

Figure 1. HPLC WATERS equipment used for the study.

2.1.4. Performance Test of HPLC WATERS Equipment

The following equipment performance tests were conducted in June 2022 (Table 1).

2.2. Method

The method of the present study was based on the optimization of a method of simultaneous determination of PHG and TPHG routinely applied by a pharmaceutical industry in Abidjan for the quantification of these in drug matrices likely to contain them. This optimization consisted in finding the best chromatography profile (best baseline, two compounds symmetrical with respect to the baseline and a short analysis time) by progressively modifying some parameters of the chromatography conditions such as: the flow rate delivered by the pump, the analysis time of the injections, the proportions of the mobile phase and the mode of preparation of the mixture.

To be achieved, we first prepared our different working solutions according to the protocol performed by the pharmaceutical industry and then the different injections were performed while using the basic chromatography conditions that

Table 1. Performance test of the WATERS HPLC equipment used for the study.

Performance test of WATERS HPLC equipment				
Pump	**Nominal flow rate**			
Test of the accuracy of the programmed flow	0.5 mL/min	2 mL/min	0.5 mL/min	2 mL/min
Average deviation Dm (%)	1.2	1.45	0.6	1.25
European Pharmacopoeia Standard 8.2: Dm < 5%	Compliant	Compliant	Compliant	Compliant
Pump Repeatability Test (n = 6)	0.5 mL/min	2 mL/min	0.5 mL/min	2 mL/min
RSD (%)	0.428		0.252	
European Pharmacopoeia Standard 8.2: RSD ≤ 0.5%	Compliant		Compliant	
Injector	**Propylparaben (PPB) peak area**			
Injector repeatability (n = 6)	1660295.33			
RSD (%)	0.72			
RSD limit (%)	1			
European Pharmacopoeia Standard 8.2: RSD < RSD limit	Compliant			
Linearity test of the injector volume (n = 5)	volume = f (PPB peak area)			
Coefficient of determination (R^2)	0.9993			
European Pharmacopoeia Standard 8.2: $R^2 > 0.99$	Compliant			
Detector	**Peak area of caffeine**			
Linearity test of the detector (n = 5)	Volume = f (Caffeine peak area)			
Coefficient of determination (R^2)	0.9994			
European Pharmacopoeia Standard 8.2: $R^2 > 0.99$	Compliant			

are those used by the pharmaceutical industry. It should be noted that all chromatograms determined during the study were obtained from the reference substances of the molecules listed in 2.1.1.

2.2.1. Preparation of the Elution Mobile Phase

The mobile elution phase consisted of the mixture of Acetonitrile/water in the proportions 80/20.

The preparation was obtained by transferring 800 mL of Acetonitrile and 200 mL of ultra pure water into a 1000 mL graduated cylinder. The mixture obtained was sonicated in an ultrasonic bath for 5 min before being transferred to a 1000 mL graduated jar (mobile phase tank).

2.2.2. Preparation of the Different Reference Solutions of PHG and TPHG

1) Preparation of the reference stock solution (RS) of PHG

Using an analytical balance and a watch glass, 38.6 mg of PHG was weighed. The weighed material was transferred to a 50 mL volumetric flask and solubilised with 20 mL of the previous mobile phase. The resulting solution was subjected to the ultrasonic bath for 10 min and then adjusted to the mark with the mobile phase to give a concentration of 0.772 mg/mL.

2) Preparation of the reference stock solution (RS) of TPHG

Using a balance, 30 mg of TPHG was weighed and then decanted into a 50 mL volumetric flask. This was solubilised and then made up to the mark with the previously prepared mobile phase. The concentration of the solution obtained was 0.600 mg/mL.

3) Preparation of the diluted Reference Solution (RS) of PHG used to perform the injection

From the previous PHG stock solution concentrated to 0.772 mg/mL, a diluted solution of 0.185 mg/mL was prepared. This was obtained by transferring 6 mL of the stock reference solution into a 25 mL volumetric flask. This was made up to the mark with the mobile phase.

4) Preparation of the diluted Reference Solution (RS) of TPHG used to perform the injection

A volume of 6 mL of the previously prepared stock solution of TPHG was withdrawn using a 1000 µL propette and transferred to a 25 mL volumetric flask. This was made up to the mark to give a solution concentration of 0.144 mg/mL.

5) Preparation of the mixture of the diluted PHG and TPHG solutions used to perform the injection

In a 50 mL volumetric flask, 1 mL of each of the diluted PHG and TPHG solutions were transferred and then made up to the mark with the mobile phase.

6) Method of preparation of the analytes according to the optimized method

In the same 50 mL volumetric flask, 38.6 mg of PHG and 30 mg of TPHG were concomitantly introduced and the whole was solubilised and adjusted with the mobile phase up to the mark.

7) Expression of results

The chromatograms were obtained from the BREEZE software of the WATERS equipment and the tables were produced by the MICROSOFT EXCEL 2016 software.

3. Results and Discussion

3.1. Chromatographic Profile and Conditions Routinely Used by the Pharmaceutical Industry

In order to obtain a better chromatographic profile of the mixture of two molecules, PHG and TPHG, previously developed by a pharmaceutical industry in Abidjan, successive optimizations of the parameters of the chromatographic conditions were performed. The parameters concerned were: the stationary phase (column), the mobile phase (the proportions of the composition), the flow rate delivered by pump and the method of preparation of the mixture of the two molecules (PHG and TPHG). To do this, we initially relied on the chromatographic conditions used by the pharmaceutical industry (basic chromatographic profile). This profile is derived from the conditions listed in Table 2. After HPLC injection, it shows three chromatographic peaks with good resolutions and a better baseline. The respective RT for each peak was 0.773 min for the first unidentified compound, 2.275 min for PHG and 7.269 min for TPHG (Figure 2). The presence of the first peak at RT = 0.773 min could be explained by a probable contamination of the stationary phase (column) and/or the equipment rinsing circuit by these same molecules or other molecules injected on a large scale in this pharmaceutical industry. The Chromatogram of the obtained mixture differs from that developed by Hasan N and al in 2012 [19] in their work on simultaneous determination of PHG and TPHG in bulk pharmaceuticals and body

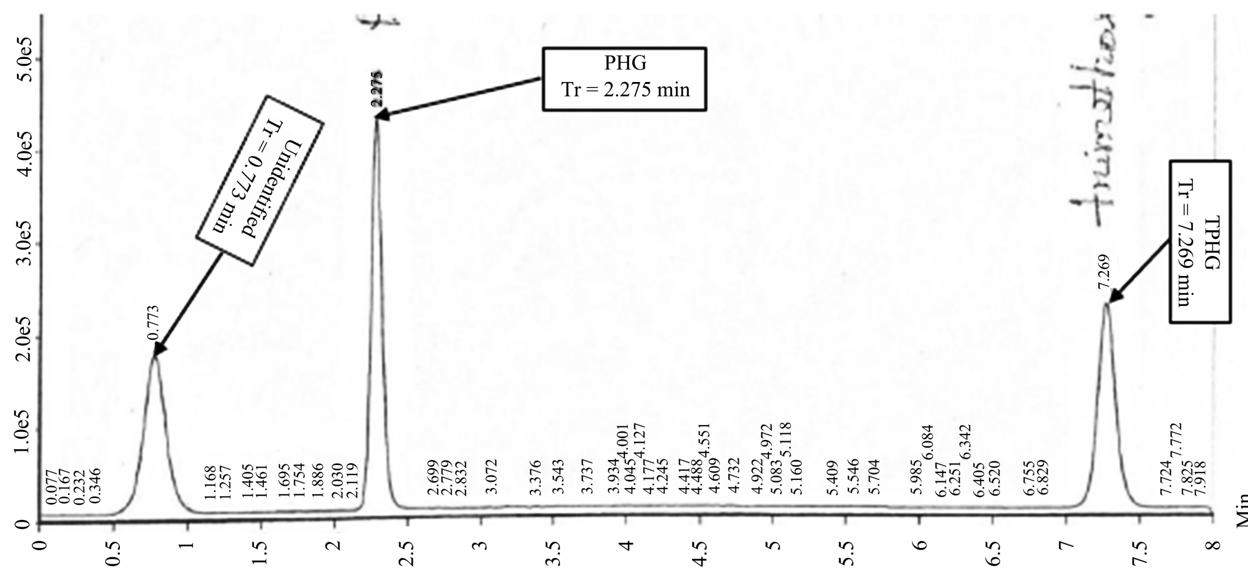


Figure 2. Chromatographic profile of the PHG and TPHG mixture obtained from the chromatographic conditions developed by the pharmaceutical industry.

Table 2. Chromatographic conditions used by the pharmaceutical industry for their various analyses.

Parameters	Data of the different parameters
Stationary phase	C18 BDS Hypersil (250 mm * 4.6 mm) 5 µm
Mobile phase (%/%)	Acetonitrile/water (60/40)
Detection wavelength (nm)	265
Flow rate (mL/min)	1
Injection volume (µL)	10
Run time (min)	8

fluids. The chromatographic profile developed by the latter identified two chromatographic peaks with good resolutions at the respective RT of 3.212 min (PHG) and 8.073 min (TPHG) and with a better baseline.

3.2. Change of the Stationary Phase

Changing the stationary phase from C18 BDS Hypersil to C18 YMC-TriArt (150 * 4.6 mm, 3 µm) while maintaining the other parameters of the chromatographic conditions resulted in the identification of a chromatographic peak corresponding to TPHG (RT = 3.274 min) in the chromatographic profile of the mixture with the presence of some background noise at the baseline (**Figure 3**). This single retention could be explained by the fact that the C18 YMC column does not contain a minimal residual silanol group (porosity = 120 Å and granulometry = 3 µm) [20], unlike the C18 BDS Hypersil column which does (porosity = 130 Å and granulometry = 5 µm) [21]. This specificity at the level of the columns could then have an impact on the polarity of the injected molecules, in particular that of PHG.

3.3. Modifying the Proportions of the Mobile Phase Composition

When the proportion of the mobile phase composition is modified (Acetonitrile 80%/Water 20%) for the successive run times of 8 min (**Figure 4(a)**) and 5 min (**Figure 4(b)**), we observe after injection at the run time of 8 min, a chromatographic profile identical to the previous one with still the only chromatographic peak of the TPHG at RT = 3, 274 min with the same background noise at the baseline level, contrary to that obtained at the 5 min analysis time, which shows two less resolved chromatographic peaks in the mixture chromatogram, each corresponding to PHG (RT = 0.979 min) and TPHG (RT = 1.504 min) with background noise at the baseline level. This difference in run times when the composition of the mobile phase is kept constant (80/20) in the first case could still be explained by the unchanged polarity of the PHG and in the second case, when the run time is reduced to 5 min, the presence of the chromatographic peak of the PHG is observed at RT = 0.979 min with an instability of the baseline. This presence of PHG at RT = 0.979 min could be explained by the fact that the

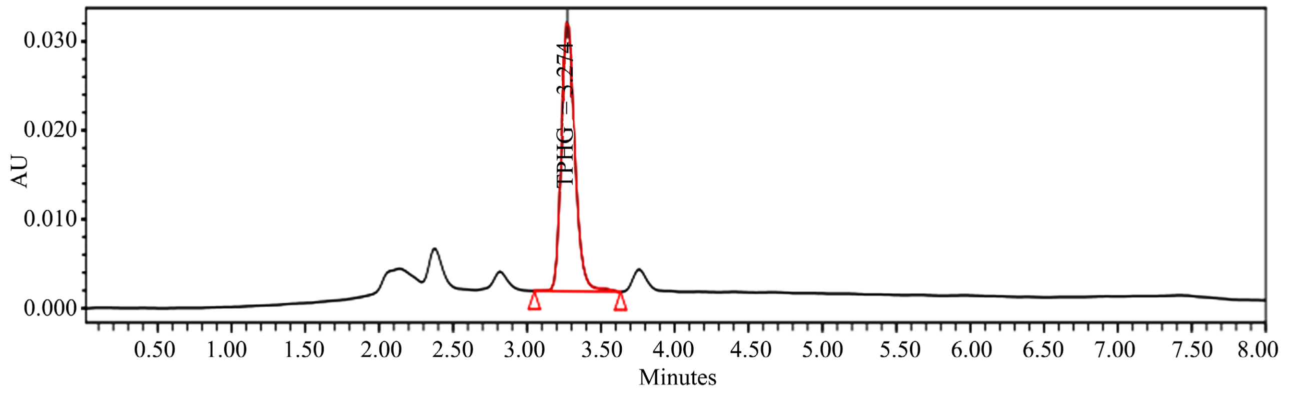
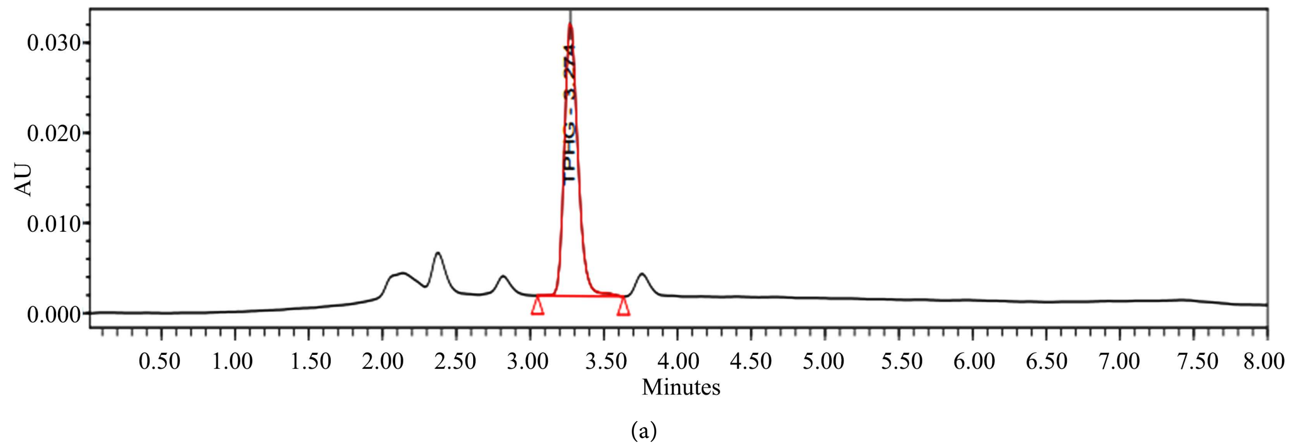
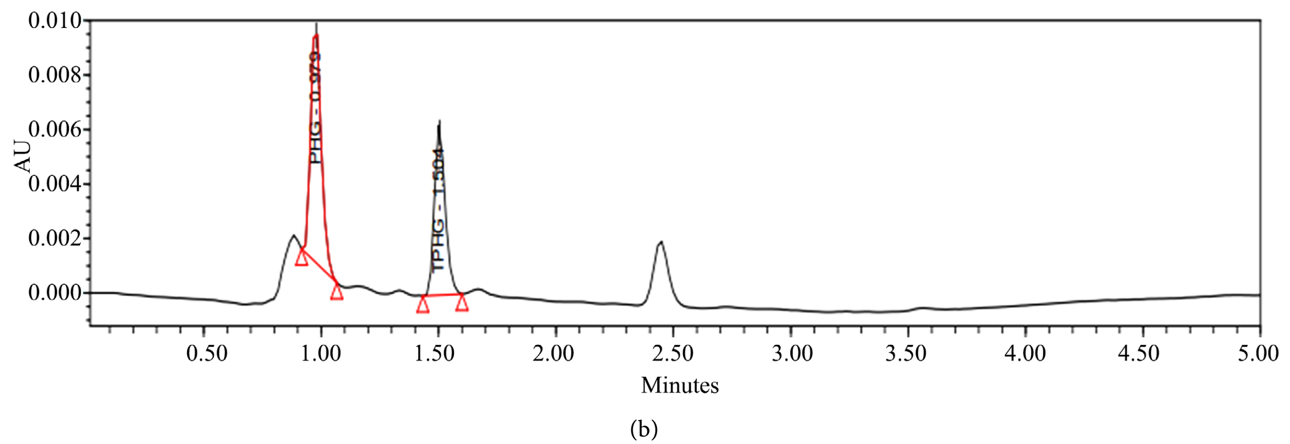


Figure 3. Chromatographic profile of the mixture obtained after changing the stationary phase.



(a)



(b)

Figure 4. Chromatographic profile of the mixture of analytes obtained after changing the proportions of the mobile phase composition at run times of 8 min (a) and 5 min (b).

increase in the proportion of Acetonitrile at the mobile phase level had an impact on the polarity of PHG, hence the presence of the latter's chromatographic peak in the chromatogram of the mixture. Our chromatographic profiles in both cases, differ from those developed by Hassan N *et al.* in 2013 [22] and Shaikh J S in 2018 [23] who worked on the simultaneous determination of two antispasmodics (PHG and TPHG) in human plasma and the study of forced

degradation of Amiloride and Furosemide in pharmaceuticals respectively. Each of the authors obtained chromatographic profiles showing two chromatographic peaks/chromatographic profile. The profile developed by [22] showed two chromatographic peaks PHG (RT = 3.215 min) and TPHG (RT = 6.344 min) for a run time of 8 min using a C18 ODS μBondapak column type (4.6 × 250 mm; 10 µm) and an elution solvent consisting of 50/50/0.3 Acetonitrile/buffer/Sulphuric acid. The one developed by [23] also showed two chromatographic peaks Amiloride (RT = 1.92 min) and Furosemide (RT = 3.14 min) for a run time of 4 min. The C18 Shim-pack type column (250 mm × 4.6 mm, 5 µm) and an elution solvent consisting of Acetonitrile/water in the proportions 65/35 was used to perform the injection. These differences could be in the proportions of the mobile phase combinations and other parameters of the chromatographic conditions.

3.4. Modification of the Flow Delivered by the Pump

When the flow rate delivered by the pump is reduced to 0.8 mL/min (**Table 3**), changes in the PHG and TPHG RT in the chromatographic profiles are observed when the run times are set to 5 min (**Figure 5(a)**) and 10 min (**Figure 5(b)**) respectively. This change in flow rate could be explained by a slow propulsion of the mobile phase and analytes within the stationary phase which will affect the detection of compounds. The flow rate used differs from that used by Raul S K *et al.* in 2015 [24] who used a flow rate of 1 mL/min when conducting their study on the simultaneous determination of Atorvastatin and Ezetimibe in pharmaceuticals. It also differs from that used in 2021 by Peleshok K *et al.* [25] in their work on the simultaneous determination of Valsartan and Atenolol by HPLC/UV in fixed dose pharmaceuticals. The flow rate used by the latter was also 1 mL/min.

Table 3. Parameters of the modified chromatographic conditions.

	Changed parameters			
Chrom. cond	**Stationary phase**	**Prop of mobilephase (%/%)**	**Flow rate (mL/min)**	**Preparation method used**
Stationary phase	**C18 YMC (150 mm * 4.6 mm) 3 µm**	C18 YMC (150 mm * 4.6 mm) 3 µm	C18 YMC (150 mm * 4.6 mm) 3 µm	C18 YMC (150 mm * 4.6 mm) 3 µm
Prop of mobilephase (%/%)	Acetonitrile/Water (60/40)	**Acetonitrile/Water (80/20)**	Acetonitrile/Water (80/20)	Acetonitrile/Water (80/20)
Flowrate (mL/min)	1	1	**0.8**	0.8
Wavelength (nm)	265	265	265	265
Injection volume (µL)	10	10	10	10
Run time (min)	8	8 & **5**	5 & **10**	10 & **6**
Preparation method used	pharmaceutical industry	pharmaceutical industry	pharmaceutical industry	**developed**
Corresponding illustration	Figure 3	Figure 4(a) & Figure 4(b)	Figure 5(a) & Figure 5(b)	Figure 6(a) & Figure 6(b)

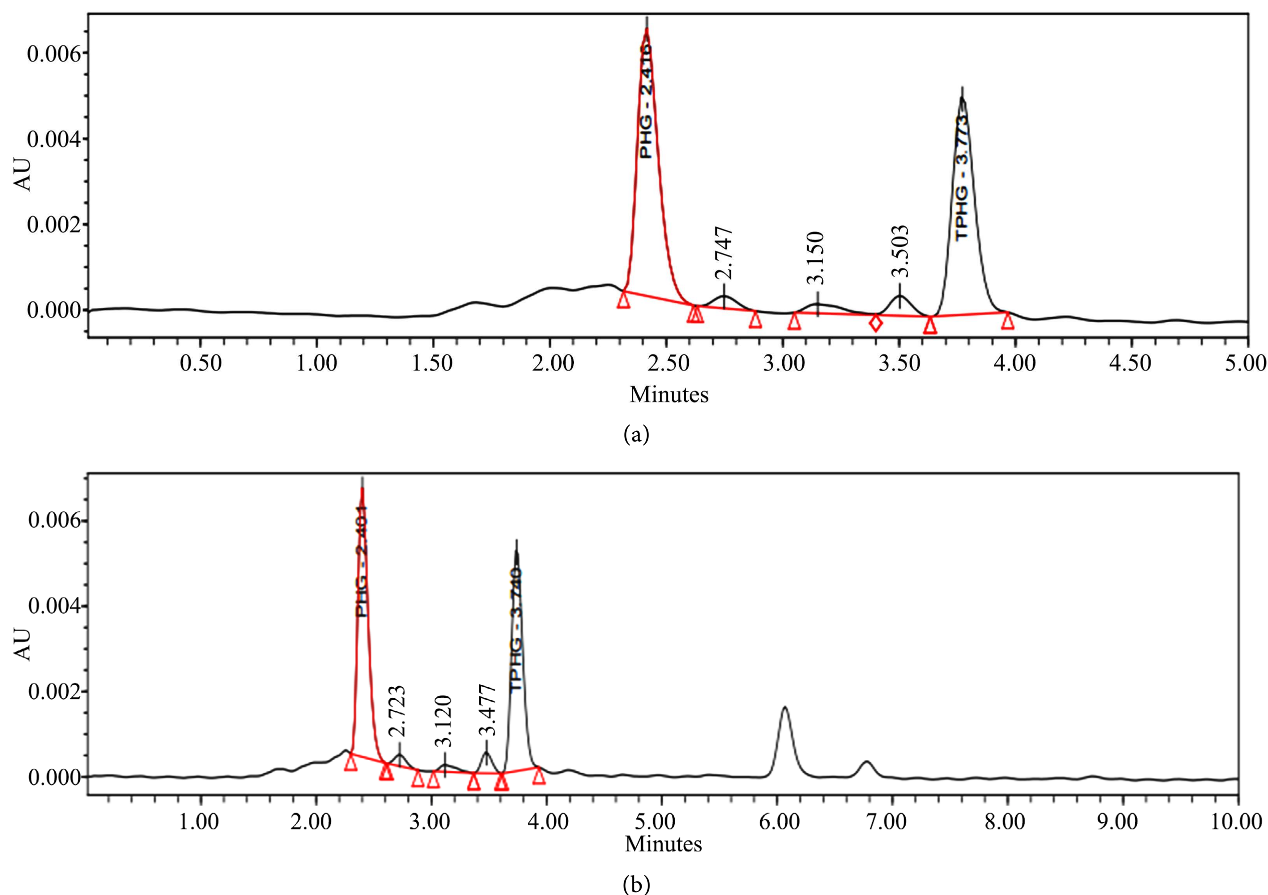


Figure 5. Chromatographic profile of the mixture of analytes obtained after modification of the flow rate delivered by the pump at analysis times of 5 min (a) and 10 min (b).

3.5. Changing the Way the Mixture Is Prepared

By modifying the method of preparation of the analytes (concomitant mixing of PHG and TPHG in the same volumetric flask), a chromatographic profile was determined that highlighted two well-separated compounds: PHG (RT = 2.391 min) and TPHG (RT = 3.735 min) at the respective run times of 10 min (**Figure 6(a)**) and 6 min (**Figure 6(b)**). The RT of these compounds are practically the same at these respective run times. This change in preparation mode contributed to the increase in analyte concentrations in the mixture, which in turn contributed to the improvement of the baseline (the eluted analyte peaks helped to overwhelm the background). The chromatographic profile obtained (**Figure 6(b)**) is similar to that determined by [24] although their chromatographic conditions are different to that used in the present study. Their chromatogram shows two chromatographic peaks corresponding to Atorvastatin (RT = 2.367 min) and Ezetimibe (RT = 3.436 min) for an elution time of 6 min. It differs from the method developed by Mahmoud SA *et al.* in 2021 [26]. This method allowed the simultaneous determination of febuxostat and diclofenac in the pharmaceutical form and human plasma by the green UHPLC technique. The latter developed a chromatographic profile that highlighted febuxostat and

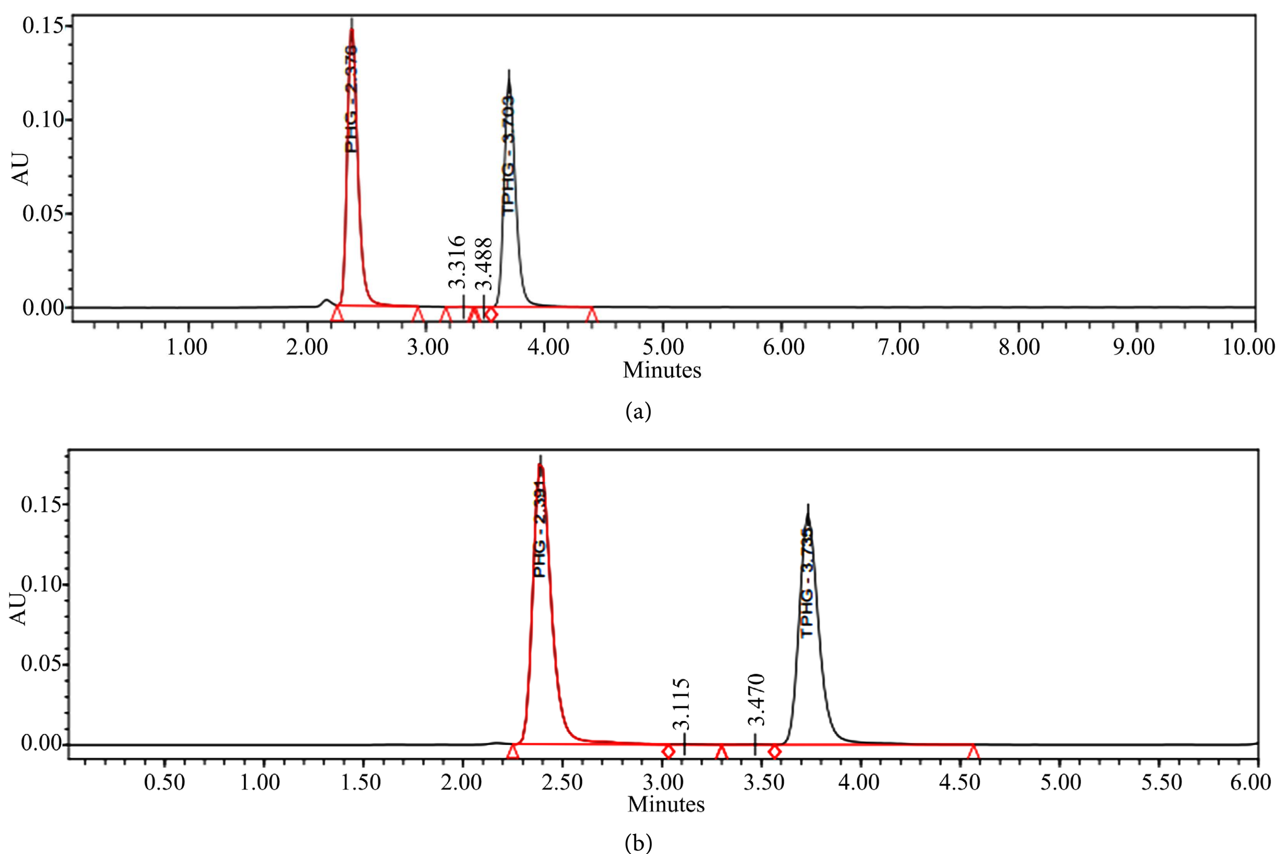


Figure 6. Chromatographic profile obtained after modification of the analyte preparation mode at the respective analysis times of 10 min (a) and 6 min (b).

diclofenac at the respective RT of 2.460 min and 4.151 min at the 10 min analysis time (profile of the mixture of febuxostat and diclofenac standards). The flow rate (1 mL/min), wavelength (280 nm) and mobile phase (Water 85/Ethanol 15) used by [26] is different from that developed in the present study.

4. Conclusions

The aim of this study was to improve a method for the simultaneous determination of PHG and TPHG used routinely by a pharmaceutical industry in Abidjan by HPLC. From the chromatographic profile used by the industry, four parameters were progressively optimized to achieve a result. These are the stationary phase, the proportions of the mobile phase composition, the flow rate delivered by the pump and the method of preparation of the mixture.

The chromatographic profile was obtained after optimisation showed two well separated chromatographic peaks corresponding respectively to PHG (RT = 2.391 min) and TPHG (RT = 3.735 min) with a stable baseline in a short run time (6 min) compared to the baseline profile that was routinely used by the pharmaceutical industry.

This optimized analytical method could be validated and applied routinely by the pharmaceutical industry for the simultaneous determination of these two

molecules in existing pharmaceutical forms (suppositories, tablets and injectable forms) with a reduced turnaround time. These actions will thus allow the industry to optimize and reduce the cost of its production.

Nevertheless, this action could be applied to other pharmaceutical products in the production chain and even be extended to the production chain of other pharmaceutical industries in Abidjan.

Acknowledgements

We would like to thank the food control department of the National Public Health Laboratory (NPHL) in Abidjan, Ivory Coast, which made this study possible.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] OMS (2018) Produits médicaux de qualité inférieure ou falsifiés.

[2] Newton, P.N., Green, M.D., Fernández, F.M., Day, N.P. and White, N.J. (2006) Counterfeit Anti-Infective Drugs. *The Lancet Infectious Diseases*, **6**, 602-613. https://doi.org/10.1016/S1473-3099(06)70581-3

[3] Bouchama, F. and Mammou, W. (2017) Validation analytique d'une méthode de dosage simultané du Paracétamol et du Tramadol dans des comprimés par HPLC. Application de la démarche harmonisée.

[4] Wertheimer, A.I., Chaney, N.M. and Santella, T. (2003) Counterfeit Pharmaceuticals: Current Status and Future Projections. *Journal of the American Pharmacists Association*, **43**, 710-718. https://doi.org/10.1331/154434503322642642

[5] Renouvellement de la commission de la transparence de la HAS 2022.

[6] Ismaili, L. (n.d.) Procédé de préparation de 1,3,5 triaminobenzene et son hydrolyse en phloroglucinol de haute pureté.

[7] Fouedjio, J., Fouelifack, F.Y., Kenfack, B., Sime, L.W., Nana, N., Temkou, S., *et al.* (2012) Efficacité du phloroglucinol sur le raccourcissement de la phase active du travail à la maternité de l'Hôpital Central de Yaoundé, Cameroun. *Clinics in Mother and Child Health*, **9**, C120502.

[8] Blanchard, C., Pouchain, D., Vanderkam, P., Perault-Pochat, M.-C., Boussageon, R. and Vaillant-Roussel, H. (2018) Efficacy of Phloroglucinol for Treatment of Abdominal Pain: A Systematic Review of Literature and Meta-Analysis of Randomised Controlled Trials versus Placebo. *European Journal of Clinical Pharmacology*, **74**, 541-548. https://doi.org/10.1007/s00228-018-2416-6

[9] Cargill, G., Salin, B., Lubin, S., Kohler, F., Coste, T. and Rautureau, J. (1992) Effet du phloroglucinol sur la réponse motrice du rectosigmoïde à l'alimentation: Étude chez des sujets atteints du syndrome de l'intestin irritable. *La Presse Médicale* (1983), **21**, 19-23.

[10] (n.d.) The United States Pharmacopoeia, USP 27, NF 22, United States Pharmacopeial Convention, Rockville, Md, USA, 2004.

[11] Tabassum, S., Afridi, B. and Aman, Z. (2005) Phloroglucinol for Acceleration of

Labour: Double Blind, Randomized Controlled Trial. *Journal-Pakistan Medical Association*, **55**, 270-273.

[12] Lecarpentier, C. (2013) La prise de médicaments au cours de la grossesse. Gynecology and Obstetrics.

[13] Direction des Activités Pharmaceutiques Cote d'Ivoire. Index Pharmaceutique 2019.

[14] Youm, I. and Youan, B.-B.C. (2013) Validated Reverse-Phase High-Performance Liquid Chromatography for Quantification of Furosemide in Tablets and Nanoparticles. *Journal of Analytical Methods in Chemistry*, **2013**, Article ID: 207028. https://doi.org/10.1155/2013/207028

[15] Tharpa, K., Basavaiah, K. and Vinay, K. (2010) Use of a Diazocoupling Reaction for Sensitive and Selective Spectrophotometeric Determination of Furosemide in Spiked Human Urine and Pharmaceuticals. *Chemical Papers*, **64**, 415-423. https://doi.org/10.2478/s11696-010-0027-5

[16] Cui, Y. and Ang, C.Y. (2002) Supercritical Fluid Extraction and High-Performance Liquid Chromatographic Determination of Phloroglucinols in St. John's Wort (*Hypericum perforatum* L.). *Journal of Agricultural and Food Chemistry*, **50**, 2755-2759. https://doi.org/10.1021/jf011304n

[17] Tatsis, E.C., Boeren, S., Exarchou, V., Troganis, A.N., Vervoort, J. and Gerothanassis, I.P. (2007) Identification of the Major Constituents of Hypericum Perforatum by LC/SPE/NMR and/or LC/MS. *Phytochemistry*, **68**, 383-393. https://doi.org/10.1016/j.phytochem.2006.11.026

[18] Slimani, S., Zalouk, T. and Zerbout, H. (2019) Mise au point et validation D'une methode de dosage de furosemide dans des comprimes de 40 mg par la chromatographie liquide a haute performance.

[19] Hasan, N., Afridi, N.S., Khan, S., Siddiqui, M.Z. and Chaiharn, M. (2012) Simultaneous Determination of Two Antispasmodic Drugs in Bulk, Pharmaceutical Products and Body Fluid by a Validated, Acetonitrile Free, Cost Effective and Stability Indicating Reverse Phase High Performance Liquid Chromatographic Method. *Analytical Chemistry*, **11**, 274-281.

[20] Brochure YMC-triart UHPLC & HPLC columns 2016.

[21] Brochure Thermo Scientific Hypersil BDS Columns Technical Guide Version 2 2010.

[22] Hasan, N., Chaiharn, M., Khan, S., Khalid, H., Sher, N., Siddiqui, F.A., *et al.* (2013) Dual Wavelength RP-HPLC Method for Simultaneous Determination of Two Antispasmodic Drugs: An Application in Pharmaceutical and Human Serum. *Journal of Analytical Methods in Chemistry*, **2013**, Article ID: 297285. https://doi.org/10.1155/2013/297285

[23] Shaikh, J.S. and Rao, N.N. (2018) Simultaneous Estimation and Forced Degradation Studies of Amiloride Hydrochloride and Furosemide in a Pharmaceutical Dosage Form Using Reverse-Phase High-Performance Liquid Chromatography Method. *Asian Journal of Pharmaceutical and Clinical Research*, **11**, 215-221. https://doi.org/10.22159/ajpcr.2018.v11i7.25783

[24] Raul, S.K., Aravelli, A.B. and Jhansi, D. (2015) RP-HPLC Method Development and Validation for the Simultaneous Estimation of Atorvastatin and Ezetimibe in Pharmaceutical Dosage Form. *Asian Journal of Pharmaceutical and Clinical Research*, **8**, 178-181.

[25] Peleshok, K., Piponski, M., Ajie, E.A., Poliak, O., Zarivna, N., Denefil, O., *et al.* (2021) Novel HPLC-UV Method for Simultaneous Determination of Valsartan and Atenolol in Fixed Dosage Form, Study of Green Profile Assessment. *Pharmacia*, **68**, 43-51.

[26] Mahmoud, S.A., El-Kosasy, A.M. and Fouad, F.A. (2021) Green UHPLC Method for Simultaneous Determination of Febuxostat and Diclofenac in Pharmaceutical Dosage Form and Human Plasma. *Journal of Analytical Sciences*, *Methods and Instrumentation*, **11**, 29-45. https://doi.org/10.4236/jasmi.2021.114004