Spectrophotometric Assessment of Benzene 1,3,5-Triol in Pure Forms and in Bulk Sample by Diazotization Coupling Reaction

Mohauman Mohammed Majeed Al-Rufaie a*, Aymen Abdul Rasool Jawad b and Lamia Abdultef Risan Al-Iessa a

a Department of Chemistry, Faculty of Sciences, Kufa University, Najaf, Iraq.
b Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Kufa University, Najaf, Iraq.

Authors’ contributions
This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information
DOI: 10.9734/JPRI/2022/v34i6B35433

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/82875

Received 22 November 2021
Accepted 27 January 2022
Published 29 January 2022

ABSTRACT

The method was published for determining microgram quantities of benzene 1,3,5-triol (phloroglucinol) in aqueous solution and some bulk samples using an easy, quick, and effective spectrophotometric method. The method uses a diazotization as well as coupling process in basic medium to produce a bright yellow water-soluble dye from Phloroglucinol and diazotized 4-Methoxy aniline That is stable and also has a maximum absorption wavelength of 420 nm. With a molar absorptivity of 1.5989×10^4 l mol^-1 cm^-1 as well as index of Sand ell sensitivity 0.001 g.cm^-2, Beer's law is followed across a concentration range of (2-40) g.ml^-1 of phloroglucinol. The ideal conditions for all color development are detailed, and even the suggested procedures for determining phloroglucinol in aqueous medium as well as some bulk sample have been successfully employed.

Keywords: Diazotization coupling; phloroglucinol; 4-Methoxy aniline; spectrophotometric assessment.

1. INTRODUCTION
The bioactive chemical phloroglucinol (1,3,5-trihydroxybenzene or 1,3,5-benzenetriol) has been utilized as a smooth muscle relaxant. Radiation-induced intracellular as well as decomposition to cellular elements like lipid, DNA, as well as protein were dramatically

*Corresponding author: E-mail: muhaimin.alrufaie@uokufa.edu.iq, mohaumanmajeed@yahoo.com;
Phloroglucinol is a yellowish white or white crystal with a melting point at 215-219°C. It is slightly water soluble, alcohol soluble, as well as ether. soluble It is employed as an antispasmodic. Its antispasmodic action is greater versus muscle than smooth muscle under spastic conditions. phloroglucinol is often used to treat colics caused by renal as well as biliary calculi, actually pain in the gastrointestinal tract or urinary tract, pain in the abdomen area of unknown origin, spastic disorders of the female genital order, and dysmenorrhea [1-4]. Phloroglucinol was a non-atropine antispasmodic drug utilized for its spasmylytic actions against the digestive and urinary tract via oral, rectal, intramuscular, or intravenous injection. Phloroglucinol is often utilized to treat aches associated with digestive system is working disorders, renal colics (pain associated with urine diseases), as well as gynecological pains. In the past, the medication was commonly used techniques women get pregnant [5-7].

By using the azo-coupling reaction in alkaline medium, the stable diazotized 4-Methoxy aniline agent has been developed to assess Phloroglucinol in aqueous solution as well as some bulk samples. The yellow outcome was computed utilizing spectrophotometry. The technique for analyzing is straight forward, quick, as well as precise. It has been successfully used in previously research to determine phloroglucinol in biological aqueous solutions and various bulk samples in the plasma of human using HPLC/mass spectrometry [8]. additionally, with gas chromatography/mass spectrometry [7,9], speed HPLC analyses of naphthodianthrones as well as phloroglucinol from Hypercom perforatum extracts are too reported [9-10] obtained by titrimetric process before another technique that has been mentioned [11-14]. these added flow injection [15] as well as reversed-phase ion-pair chromatography [16].

2. MATERIALS AND METHODS

2.1 Devices

The UV-Visible 160 electronic double-beam calculating spectrometer was used for every spectral as well as absorbance investigations. Sensitive balance (Sartorius BL210S) (Germany)

Bath water.

2.2 Material and Reagents

Every chemicals employed are of the greatest purity possible as well as have not been purified further.

2.2.1 Solution of benzene 1,3,5-triol 500 g.ml⁻¹

0.05g of purified chemical BDH is dissolved in 100 ml 100% ethanol. The bulk sample solutions are made using the same process and then moved to a a black bottle containing they will be for at least one week. Fluka Chemical Material Company submitted another bulk sample of benzene 1,3,5-triol with a purity of 98 percent.

2.2.2 Diazotized 4-methoxy aniline 1x10⁻²M reagent solution

Taken and dissolved 0.003 gm of purified 4-methoxy aniline Fluke in deionized water, then introducing 1 M HCl BDH with volume 1 ml and shaking well, accompanied by 0.025 M sodium nitrite BDH with volume 2 ml as well as shaking completely, then diluting to 25 ml and cooling to around 5°C for 30 minutes.

2.3 Methodology

A sequence of volumetric flasks 25 ml containing to increasing volumes of phloroglucinol 500 g.ml⁻¹ from the working standard solution were transmitted to support the concentration range 2-40 g.ml⁻¹ in finished dilution, then 0.1ml of 1 M solution sodium hydroxide as well as 2 ml diazotized 4-methoxy aniline agent 0.001 M solution were increased as well as watered down to the mark with deionized water, well integrated. and let aside at the temperature of room for 20 minutes, at 420 nm, the absorbance of the yellow dye produced was compared to a blank sample that included all components excluding phloroglucinol. It was decided to create a calibration curve [17,18].
3. RESULTS AND DISCUSSION

Investigation of the optimum reaction conditions: the effects of a variety of variables on the optical properties of an azo dye characteristics have been investigated, as well as the experimental conditions have been adjusted.

3.1 Reagent Volume Impact

The impact of diazonium agent \(0.001 \text{M}\) with volume between \(0.1 - 5\) ml on absorbance ferocity was investigated, as well as \(2.5\) ml was revealed to be control Fig. 2.

3.1.1 Acid Impact

It was discovered that the existence of acid enhanced the ferocity for the output composed, so various acids including \(\text{HNO}_3\), \(\text{H}_2\text{SO}_4\), \(\text{CH}_3\text{COOH}\), and \(\text{HCl}\) were investigated, and it was discovered that every one of the studied acids gave nearly the same intensity, for that \(\text{HCl}\) was chosen, and it was discovered that \(1.5\) ml for \(1\text{M} \text{HCl}\) gave a high sensitivity especially in comparison to those other acids, so it was chosen in following experiments [19].

Table 1. Influence of HCl acid volume

| Volume of acid | Absorbane |
|---------------|-----------|
| 0.5           | 0.442     |
| 1             | 0.674     |
| 1.5           | 0.771     |
| 2             | 0.768     |
| 2.5           | 0.764     |
| 3             | 0.766     |

3.1.2 Base impact

Because the dye generated in an alkaline medium grew more intense and stable, the influence of various alkaline solutions on the colored result was investigated, including sodium carbonate, potassium hydroxide, ammonium hydroxide, sodium acetate, and sodium hydroxide . Only when the reaction was performed in the sodium hydroxide presence did it achieve maximum sensitivity and stability. The impacts of different \(\text{NaOH}\) concentrations \(0.1-4\) M were investigated, with \(1\) M appearing to be the best. The impact of several quantities of \(1\) M \(\text{NaOH}\) was also investigated, ranging from \(0.05\) to \(4\) ml, with \(0.2\) ml being the most effective.

3.2 Effect of Order of Addition

The optimal order of additions for the maximum absorption was discovered to be \((\text{PHI}+\text{B}+\text{R})\) where \((\text{PHI}=\text{Phloroglucinol material, B=base, as well as R=reagent})\) which was used in further studies.

3.2.1 Temperature impact

The proposed method’s finished product was investigated at various temperatures. The analysis shows that in the temperature range \((0-80)\) °C, the absorbance values remain virtually constant, however at greater temperatures, the amount of absorbance decreases, signaling the breakdown of a product due to protracted heating. At room temperature \(20\) °C, the colorful product remained steady. As a result, in this procedure, room temperature is chosen. [20].

3.2.2 Reaction time impact

After 20 minutes, the color ferocity acquired its maximal after the phloroglucinol was interacted instantly with the agent solution. As a result, a 20 minute development time was chosen as the best option in the overall method. For at least 3 hours, the color acquired remained steady.

The best experimental conditions for identifying phloroglucinol have been identified. The Diazonium reaction took place in an acidic medium [8], and \(1\) M hydrochloric acid was chosen [9], as well as the dye generated grew more powerful additionally an alkaline medium stable [10].

3.2.3 Absorption spectra

Whenever a dilute solution of Phloroglucinol is coupled with diazotized 4-Methoxy aniline, the yellow colored dye is produced instantaneously. Under the above-mentioned conditions, This indicates maximum absorption, in contrast to the colored reagent blank, which shows no absorption at maximum absorption at 420 nm (Fig.4). The absorption spectra are displayed. The maximum absorption wavelength of 420 nm is still used in later studies.

3.2.4 Calibration curve

Under a correlation value of 0.9990 as well as an intercept of 0.0379, a linear relationship between the concentration and absorbance of Phloroglucinol was seen under perfect experimental circumstances spanning the concentration range of \(2-40\) \(\mu\text{g.ml}^{-1}\) (Fig 5). Above \(40\) \(\mu\text{g.ml}^{-1}\) of phloroglucinol, a negative divergence from Bear’s law was detected. The
molar absorptivity was $5.2164 \times 10^3$ l.mol$^{-1}$.cm$^{-1}$. And Table (2) appeared Analytical characteristics of the Phloroglucinol assessment technique established.

![Chart Title](chart.png)

**Fig. 2. Influence of regent volume**

![Absorbance](absorbance.png)

**Fig. 3. Influence of temperature on the color product**

![Absorption spectra](spectrum.png)

**Fig. 4. Absorption spectra**

A: Phloroglucinol (20 μg /ml) with 4-Methoxy aniline $1 \times 10^{-2}$ M color product instead of reagent blank; B: Reagent blank versus deionized water; C: Pure Phloroglucinol versus absolute ethanol
3.2.5 Precision and accuracy

Phloroglucinol was measured at three distinct concentrations to establish the calibration graph's accuracy and precision. Table (3) means that the data are exact and accurate enough.

3.2.6 Nature of product and reaction mechanism

Job's technique of the continuous of variations additionally also the ratio mole technique have been unitized to determine the structure (ratio of phloroglucinol to diazotized 4-methoxy aniline agent) of the yellow azo dye produced. The dye was created by reacting phloroglucinol with diazotized 4-methoxy aniline agent in a 1:1 ratio, according to the findings, (Figs. 6&7), demonstrating a mono azo dye that adopts the following scheme.

The azo dye's supposed stability constant in aqueous solution under the circumstances $2.16 \times 10^6$ l. mole$^{-1}$ has been calculated under the conditions of the experimental procedure.

The derived regression equation, as well as the procedure's analytical properties, are given in (Table 3) [21,22].

![Calibration curve of Phloroglucinol](image)

**Scheme 1. Color product formation**
Table 2. Analytical characteristics of the phloroglucinol assessment technique established

| Analytical parameters                  | Present method                  |
|----------------------------------------|---------------------------------|
| The equation of regression             | Y = 0.0414X + 0.1269            |
| Linearity (μg ml⁻¹)                    | (2-40)                          |
| Correlation coefficient, r²            | 0.9990                          |
| Limit of detection LOD (μg ml⁻¹)       | 0.598                           |
| LOQ (μg ml⁻¹)                          | 1.451                           |
| (RSD Average) %                        | 0.956                           |
| Recovery Average %                     | 100.823                         |
| Molar absorptivity (l mol⁻¹ cm⁻¹)      | 5.2164×10³                      |
| The sensitivity of Sandell (μg cm²)    | 0.001                           |

Table 3. Shows the proposed method's precision and accuracy

| No. | Conc. of Phloroglucinol μg ml⁻¹ | Error %* | Recovery* | R.S.D %* |
|-----|---------------------------------|----------|-----------|----------|
|     | present                         | found    |           |          |
| 1   | 2                               | 1.97     | 98.500    | 1.226    |
| 2   | 20                              | 20.33    | 1.650     | 101.650  | 0.932    |
| 3   | 40                              | 40.53    | 1.320     | 101.320  | 0.712    |

* Average for five determinations

Table 4. The use of the suggested as well as authorized techniques for assessing phloroglucinol in bulk sample

| Phloroglucinol bulk Samples | Deliberated method | Standard method | Official Values (t),(F) |
|-----------------------------|--------------------|-----------------|------------------------|
|                             | Recovery %         | RSD%            | Recovery %             | RSD%                  | 2.732   |
|                             |                    |                 |                        |                       | (F) Value = 19.0 |
|                             |                    |                 |                        |                       | 1.023   |
|                             |                    |                 |                        |                       | (t) Value = 4.303 |
| Phloroglucinol pure         | 100.490            | 0.956           | 100.560                | 1.071                 | 2.732   |
| Phloroglucinol 98%          | 100.910            | 1.029           | 101.330                | 1.110                 | 1.023   |

* Average for five determinations

Fig. 6. Continuous variation plot

3.2.7 The technique’s applicability

The proposed methodology are already used to detection and quantification of Phloroglucinol in aqueous solutions, and two sample of bulks containing it were examined, as well as they provided good precision and accuracy, as indicated in the table (Table 3). The proposed method was found to be superior than the standard method. As indicated in table, the
study’s findings were statistically compared to the conventional strategy utilizing a variation precision ratio (F-test) additionally precision student (t-test) in the middle of confidence (95 percent) with two degrees of freedom. The F-test and T-test results were lesser than the theoretical amount ($F=19.0$, $t=4.303$). The amounts of ($F = 2.732$) as well as ($t =1.023$) were also lesser than the theoretical amount ($F=19.0$, $t=4.303$) for the technique under investigation. There was no significant contrast identified between the researched approach and the standard strategy (average of five examinations). Furthermore, as shown in Table 3, the approach under consideration is suitably compared to several previously described techniques. displaying the texts, corporate brand names, phloroglucinol identification in bulk samples using the approved procedure, and recovery of the Phloroglucinol in the suggested approach [4,23-25].

4. CONCLUSIONS

Depending on its diazotized conjecting reaction with 4-methoxy aniline, a quick, quick, efficient, extremely accurate spectrophotometric technique for determining trace quantities of Phloroglucinol in aqueous solution has been devised, which does not require temperature control or solvent extraction.

ACKNOWLEDGEMENTS

The author gratefully acknowledges the financial support by tertiary educational fund, Kufa University, faculty of science and chemistry department, Iraq.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Martindale W. Martindale. The extra pharmacopoeia. In J.E.F. 31st Edition. London: Royal Pharmaceutical Society. 1996;44-45.
2. Budavari S. The Merck index: an encyclopedia of chemicals, drugs and biologicals. In M.J.O’ Neil, A. Smith and P.E. Heckelman.13th ed. Whitehouse Station, N.J.: Merck. 2001;7-8.
3. United States Pharmacopoeia Convention, Inc., The United States Pharmacopoeia USP 27: The National Formulary: NF 22, Washington, D.C.,USA. 2000;233-235.
4. Stationary Office (Great Britain). British Pharmacopoeia. London: Stationary Office. 2009;4476-4478.
5. Sasaki, D. Kido, A. and Yoshida, Y., Effect of antispasmodic drugs on the colonic motility. II clinical study in man, International Journal of Clinical Pharmacology Therapy and Toxicology. 1984;22(7):338–341.
6. Tabassum S, Afridi B, Aman Z. Phloroglucinol for acceleration of labour:
double blind, randomized controlled trial, Journal of the Pakistan Medical Association. 2001;55(7):270–273.

7. Louvel D, Delvaux M, Staumont, G., Intracolonic injection of glycerol: A model for abdominal pain in irritable bowel syndrome, Gastroenterology. 1996;110(2): 351–361.

8. Cargnin ST, De Matos Nunes J, Haas JS. Supercritical fluid extraction and high performance liquid chromatographic determination of benzopyrans and phloroglucinol derivative in Hypericum Polyanthemum. Journal of Chromatography B. 2009;878(1):83–87.

9. Cui Y, Ang CYW. Supercritical fluid extraction and high-performance liquid chromatographic determination of phloroglucinol in St. John’s worth (Hypericum perforatum L). Journal of Agricultural and Food Chemistry. 2002;50 (10):2755–2759.

10. Tolonen A, Hohtola A, Jalonen J. Fast high-performance liquid chromatographic analysis of naphthodianthrones and phloroglucinol from Hypericum perforatum extracts. Phytochemical Analysis. 2003;14 (5):306–309.

11. Kim H, Roh H, Lee HJ. Determination of phloroglucinol in human plasma by high-performance liquid chromatography-mass spectrometry, Journal of Chromatography B. 2003;792(2):307–312.

12. Bilia AR, Bergonzoli MC, Mazzi G, Vincieri FF. Analysis and stability of the constituents of Artichoke and St. John’s wort tinctures by HPLC-DAD and HPLC-MS, Drug Development and Industrial Pharmacy. 2002;28(5):609–619.

13. Lartigue-Mattei C, Lauro-Marty C, Bastide M. et al. Determination of phloroglucinol in human plasma by gas chromatography-mass spectrometry. Journal of Chromatography. 1993;617(1):140–146.

14. Amin D, Bashir WA. Titrimetric determination of phenol resorcinol and phloroglucinol, Talanta. 2004;61(4):283–284.

15. Xie CG, Li HF. Determination of phloroglucinol by a new flow injection Chemiluminescence method, Guang Pu Xue Yu Guang Pu Fen Xi. 2004;24(12):1521–1523.

16. Hasan N, Afridi NS, Khan S, Siddiqui MZ, Chauhan M. 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. 2012;11(7): 274–281.

17. Al-Rufaie, M.M.; Al-Sharefy, A.N.; Kathem, K.H. New spectrophotometric method for the determination chlorpromazine hydrochloride in pharmaceutical preparations by using oxidative coupling reaction. Inter. J. Univ. Pharm. Bio. Sci. 2013;2(4):19–29.

18. Al-Rufaie MM. New spectrophotometric method for the determination of Sulfamethoxazole drug, World Journal of Pharmacy and Pharmaceutical Sciences. 2016;5(3):172-180.

19. Al-Rufaie MM, Jawad AA, Sadiq HM. Colorimetric estimation for salbutamol sulphate in pure form and in different types of pharmaceutical. Int. J. Chem. Tech. Res. 2016;9(11):432-440.

20. Hanaa KAT, Al-Rufaie MM, Zahraa YM. Spectrophotometric determination of metoclopramide medicine in bulk form and in pharmaceuticals using orcinol as reagent. Ovidius Univ. Ann. Chem. 2019;29(2):85-93.

21. Al-Rufaie MM. Modern kinetic spectrophotometric procedure for estimation of furosemide drug as bulk form and in pharmaceuticals preparations. Curr. Issues Pharm. Med. Sci. 2016;29(4):184-190.

22. Ahmed AZ, Al-Rufaie MM. Determination of trace amount of chlorpromazine hydrochloride in its pure form and in Pharmaceutical Preparations by using spectrophotometric analysis, Sapporo Medical Journal. 2021;55(3):398-406.

23. Al-Rufaie MM, Al-labban HMY, Salih NS. Reduction and assessment of chloramphenicol antibiotic as pure from and in various kinds of pharmaceuticals by utilizing spectrophotometric approach, Iranian Journal of Organic Chemistry. 2017;9(2):2087-2092.

24. Barrak MH, Al-Rufaie MM, Motaweq ZY. Thymol quantitative analysis in medicinal formulation types through employing of nano-technology and antimicrobial activity
in some pathogenic bacterial isolates,

25. Gary D. Christian., Analytical Chemistry., 6 Nusantara Bioscience. 2021;13(1):129-137.

© 2022 Al-Rufaie et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/82875