. ***Original Research Article***

SPECTROPHOTOMETRIC ASSESSMENTOFBENZENE 1,3,5-TRIOL IN PURE FORMS AND IN BULK SAMPLE BY DIAZOTIIZATION COUPLING REACTION

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 $1.5989x\ 10^{4}$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

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 reduced by phloroglucinol. A phenolic chemical is Phloroglucinol.

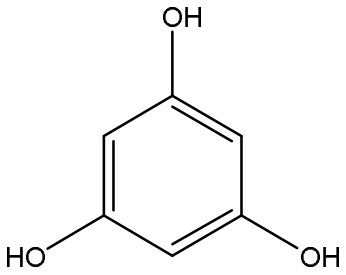


Figure 1. Phloroglucinol chemical structure

Phloroglucinol is a yellowish white or white crystal with a melting point at 215-219^{o}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 spasmolytic 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 processbefore another technique that has been mentioned [11-14]. these added flow injection [15] as well as reversed-phase ion-pair chromatography [16].

Materials and Methods

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

Material and reagents

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

Solution of benzene 1,3,5-triol500 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.

Diazotized 4-methoxy aniline $1x10^{-2}$M 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.

Methodology

A sequence of volumetric flasks 25ml containing to increasing volumes of phloroglucinol 500 $g.ml^{-1}$ from the working standard solution were transmitted to support the concentration range2-40$g.ml^{-1}$ in finished dilution, then 0.1mlof 1M solution sodium hydroxide as well as 2 ml diazotized 4-methoxy aniline agent 0.001Msolution 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].

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.

Reagent volume impact

The impact of diazonium agent 0.001M with volume between 0.1-5 ml on absorbance ferocity was investigated, as well as 2.5 ml was revealed to be control. **Figure 2.**

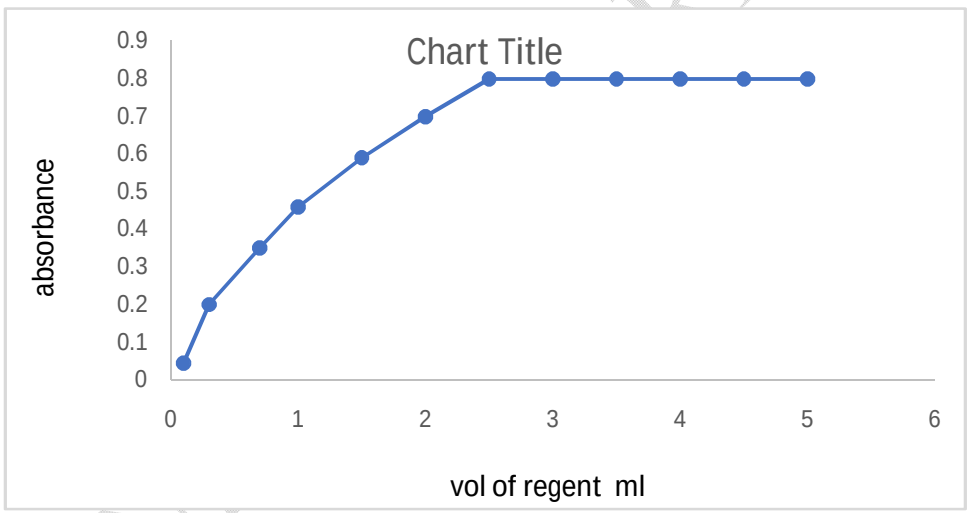


Figure 2. influence of regent volume

Acid impact

It was discovered that the existence of acid enhanced the ferocity for the output composed, so various acids including HNO_3, H_2SO_4, CH_3COOH, and HCl

wereinvestigated, and it was discovered that every one of the studied acids gave nearly the same intensity, for that HCl was chosen, and it was discovered that 1.5 ml for 1M HCl gave a high sensitivity especially in comparison to those other acids, so it was chosen in following experiments [19].Table 1**.**

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**

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 NaOH concentrations 0.1-4 M were investigated, with 1 M

appearing to be the best. The impact of several quantities of 1 M NaOH was also investigated, ranging from 0.05 to 4 ml, with 0.2 ml being the most effective.

Effect of Order of Addition

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

Temperature impact

The proposed method's finished product was investigated at various temperatures. The analysis shows that in the temperature range (0-80) ^{o}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 ^{o}C, the colorful product remained steady. As a result, in this procedure, room temperature is chosen. [20].Figure 3.

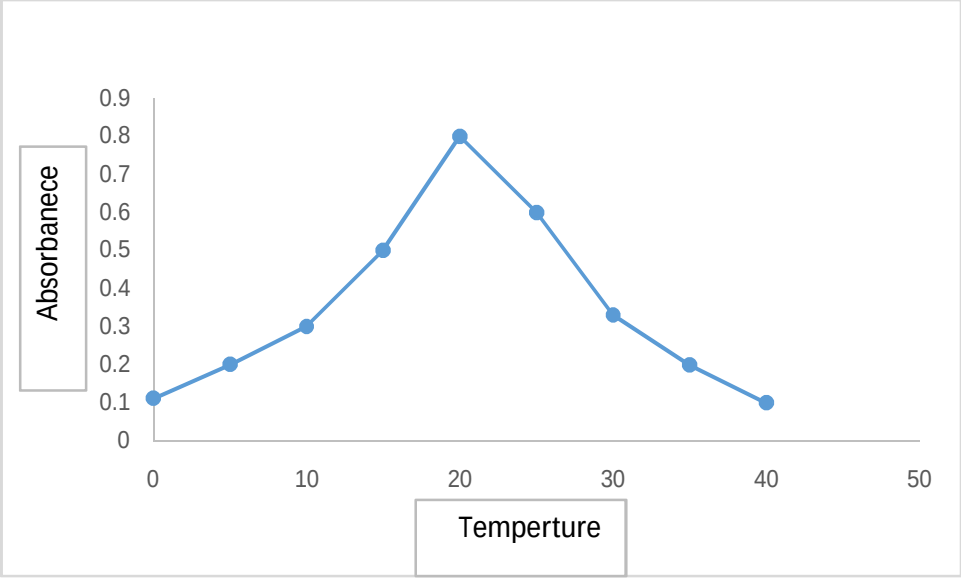


Figure 3. influence of temperature on the color product

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 20minute 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

Diaz onium reaction took place in an acidic medium [8], and 1M hydrochloric acid was chosen [9], as well as the dye generated grew more powerful additionally an alkaline medium stable [10].

Absorption spectra

When ever 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 .(Figure.4) The absorption spectra are displayed. The maximum

absorption wavelength of 420 nm is still used in later studies.

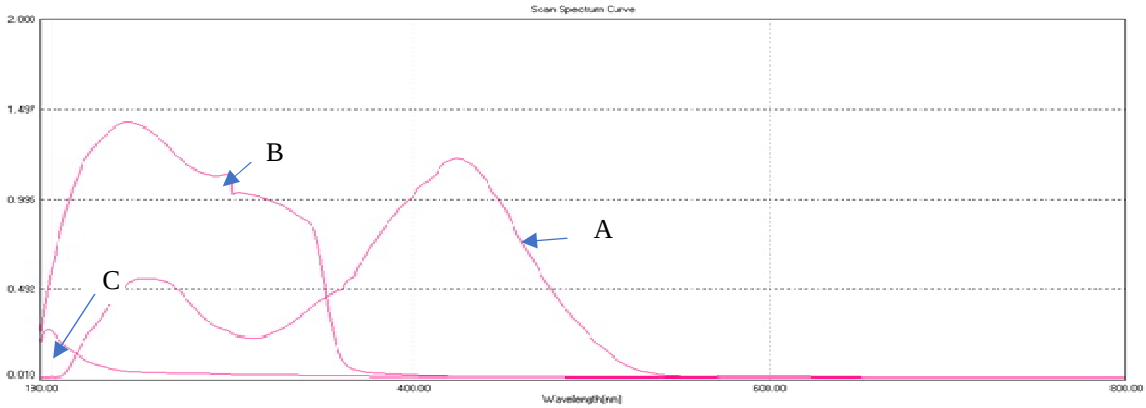


Figure4. Absorption spectra

A : Phloroglucinol (20 µg /ml1) with 4- Methoxy aniline $1x10^{-2}$ M color product instead of reagent blank.

B : Reagent blank versus deionized water C: Pure Phloroglucinol versus absolute ethanol

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 ofPhloroglucinolwas seen under perfect experimental circumstances spanning the concentration range of 2-40 $\mu g.ml^{-1}$ (Figure.5). Above 40 $\mu g.ml^{-1}$ of phloroglucinol, a negative divergence from Bear's law was detected. The molar absorptivity was 5.2164×10^{3} $l.mol^{-1}.cm^{-1}$. And table (2) appeared Analytical characteristics of the Phloroglucinolassessment technique established.

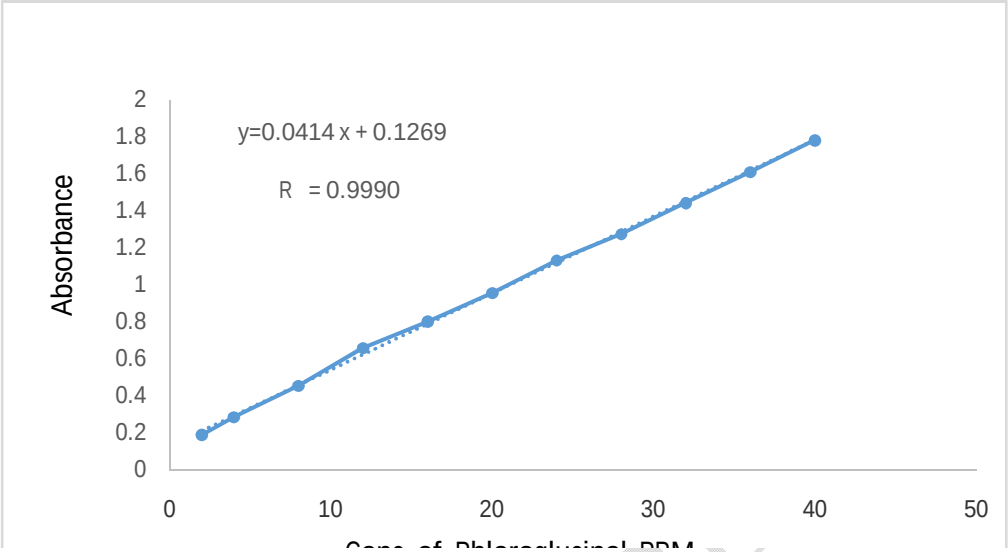


Figure 5. Calibration curve of Phloroglucinol

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, r^2	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 ($l\ mol^{-1}\ cm^{-1}$)	5.2164×10^{3}
The sensitivity of Sandell ($\mu g\ cm^{-2}$)	0.001

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**.**

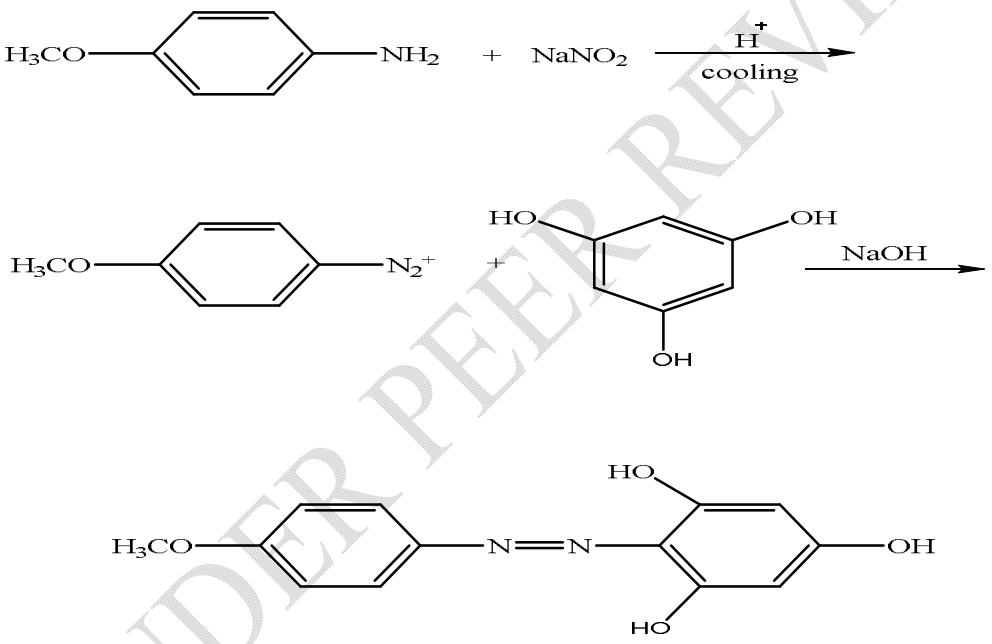
Table 3.Shows the proposed method's precision and accuracy.

No.	Conc. of Phloroglucinol µg ml^{-1}		Error %*	Recovery*	R.S.D %*
	present	found			
1	2	1.97	- 1.500	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 assessments

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., (Figure.6&7), demonstrating a mono azo dye that adopts the following scheme:



Colored Azo dye

Scheme 1. color product formation

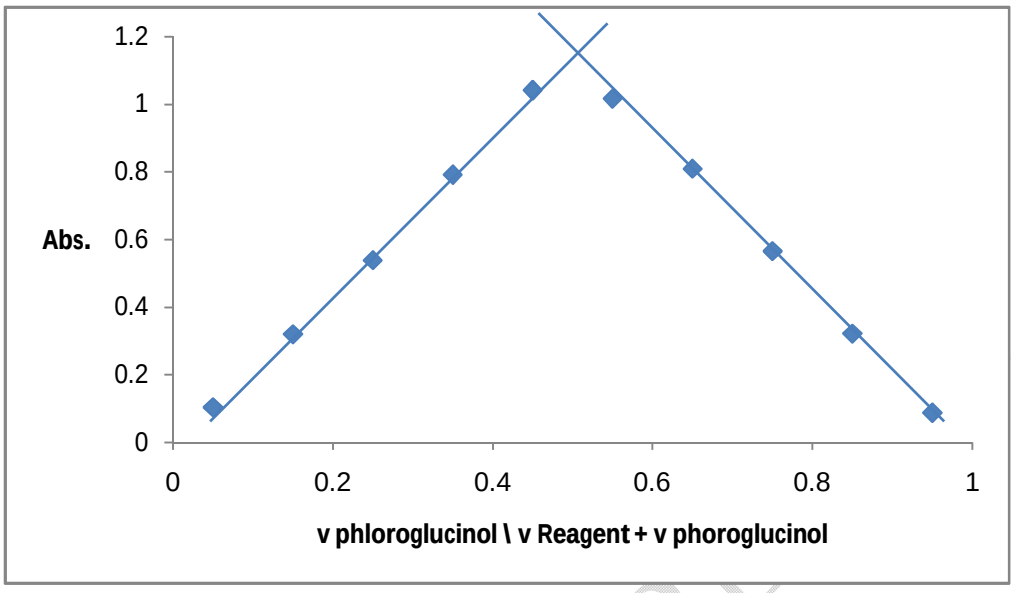


Figure6. Continuous variation plot

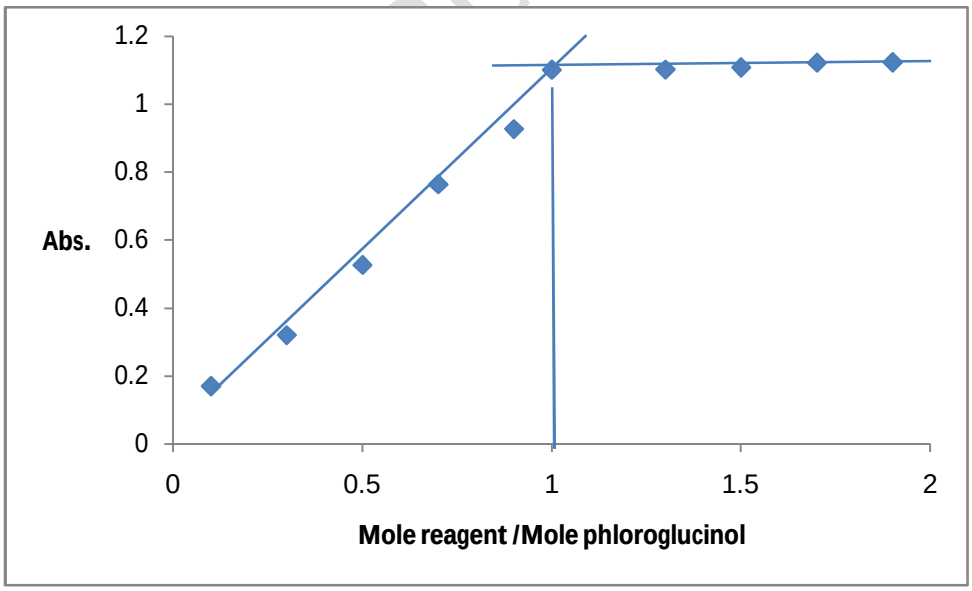


Figure7.Mole ratio plot

The azo dye's supposed stability constant in aqueous solution under the circumstances2.16×10^{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].

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 (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].

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 %**	
Phloroglucinol pure	100.490	0.956	100.560	1.071	2.732
Phloroglucinol 98%	100.910	1.029	101.330	1.110	**(**F)Value = 19.0 1.023 (t)Value=4.303

* Average for five determinations

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.

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