**Journal of Medical and oral biosciences**
**ISSN (Online): 3007-9551**
**ISSN (Print): 3007-9543**

**JMOB**
**Open Access DOAJ**

**TYPE Research Article**
**Published: 20/ 06 / 2024**

# Biochemical Analyzer and Ultra violet visible Spectrophotometer used for the estimation Guaifenesin and Theophylline in blood samples

Allaa Hussein Mhdi [1]

[1] Al-Iraqia University College of Dentistry/ Department of Basic Science Chemistry Lab/ Baghdad/ Iraq
ORCID https://orcid.org/my-orcid?orcid=0000-001-9108-8076

**OPEN ACCESS**

**ARTICLE INFO**
Received: 15/ 04 / 2024
Revised: 15/ 05 / 2024
Accepted: 03/ 06 / 2024
Publish online: 20/ 06/ 2024

* Corresponding Author email address : allaa.hussein1@gmail.com
https://orcid.org/my-orcid?orcid=0000-001-9108-8076

**CITATION**

Allaa Hussein Mhdi. (2024). Estimation Of Blood Guaifenesin And Theophylline Using Biochemical Analyzer And Ultra Violet Visible Spectrophotometer. JMOB. 1; (1): 18-29.

DOI: https://doi.org/10.58564/jmob.34



## Abstract

Guaifenesin (GU) and Theophylline (THP) are combined to treat asthma, chronic bronchitis, and emphysema symptoms, and ameliorating various respiratory conditions like shortness of breath, wheezing and cough. This study intended to investigate the effects of Guaifenesin and Theophylline on the level of CK-MB and LDH enzymes using a Biochemical Analyzer and Spectrophotometer and their impact on clinical conditions. Random serum samples were collected from patients aged 40 to 45 years with varying drug doses (concentrations). The levels of CK-MB and LDH were measured according to suggested methods. The results of this study showed that an increase in the concentration of each drug and their combination led to elevated CK-MB activity. Conversely, an increase in drug concentration resulted in a decrease in LDH activity.

**In conclusion,** this study investigates the relationship between the concentration of Guaifenesin (GU) and Theophylline (THP) and the level of CK-MB and LDH in treated patients that were estimated by Biochemical Analyzer and Ultra violet visible Spectrophotometer.

**Keywords:** Biochemical analyzer, Guaifenesin, Theophylline, UV-Visible spectrophotometer

## Introduction

A biochemistry analyzer is used for quantitative measurements and analyzing chemical and biological compounds in humans. Its rapid, accurate, and reproducible analysis of the quality and composition of RNA, DNA, and protein samples with exceptionally small sample quantities and many advantages over gel electrophoresis methods. The application includes determining components in different samples like metal complexes, fluid bodies, drug

analysis, and pharmaceutical analysis (1,2,3). Guaifenesin (Figure 1) and Theophylline (Figure 2) are drugs used to treat respiratory hypersensitivity reactions, such as chronic bronchitis, asthma, and emphysema. The drugs ameliorate various symptoms, such as cough, shortness of breath, and wheezing.

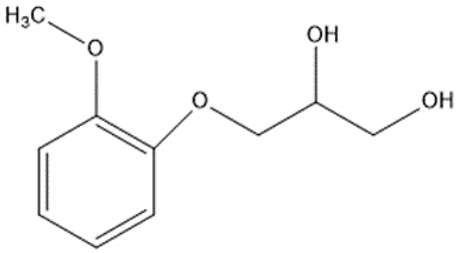


Figure .1. Chemical structure of guaifenesin drug

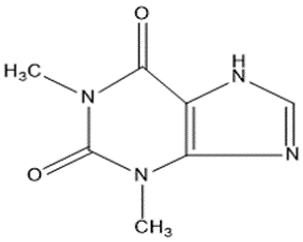


Figure .2.Chemical structure of Theophylline drug

These drugs act by widening the bronchia and increasing the flow of air in the lungs (4,5,6). A review of the literature on the effects of Guaifenesin and Theophylline on the blood level of CK-MB and LDH enzymes revealed scarce previous publications. Consequently, this work was designed to study the effect of Theophylline and Guaifenesin on the level of CK-MB and LDH in forty-year-old patients.

## Materials and Methods

### Sample collection

The blood samples were collected in plain test tubes (without anticoagulant) from eight people, including four healthy people and four patients treated with Theophylline and Guaifenesin medications. The blood samples were centrifuged for ten minutes at 3000 RPM to separate serum. The serum was collected and kept at -20 for further analysis.

### Measurement of enzymes

1. Lactate dehydrogenase (LDH) was measured according to the method described previously by Justyna *et al.*, (2023). This method includes the following:

- Reagent A: LDH volume = 10/40/80 ml Buffer 80 mmol/L, Sodium chloride 200 mmol/L, and Sodium pyruvate 1.6 mmol/L.
- Reagent B: LDH volume = 10/20 ml.

2. Creatine kinase-MB (CK-MB) measurement included the following:

- R1. Reagent (1 x 20ml): Buffer/Glucose/NAC. Imidazole buffer 100 mmol/L pH 6.7, glucose 20 mmol/L, NAC 20 mmol/L, magnesium acetate10 mmol/L, NADP 2.5 mmol/L,HK 4 KU/L, EDTA 2 mmol/L.
- R2. Reagent(1 x 5 mL): Substrate/Coenzymes. CP 30 mmol/L,AMP 5 mmol/L, ADP 2 mmol/L, di(adenosine-5´) pentaphosphate 10 mol/L, G6PDH 1.5 KU/L. Sufficient CK-
- M human antibody to inhibit ≥ 3000 U/L of CK-MB at 37ºC.

**Pure standard( GU and THP ) preparation**

- About 0.02 g of the purified material (Theophylline and Guaifenesin) was dissolved in 10 ml and then diluted to 100 ml into 100 ml with distilled water. From 200 µg.ml-1, 5 concentrations of the solution (10, 30, 50, 70, 100 µg/ $ml^{-1}$) were prepared by using the low dilution ( the preparation done by solubility testing).
- Pharmaceutical tablets for ( GU and THP ) preparation (7,8). In the same conditions method used for pure standard, the total weight of one pill of GU tablets containing 600 mg of commercial drugs was used. The average weight was calculated and dissolved. After shaking well, filtered and put into a 25 ml volumetric flask. The residue has been CAN washed, and the amount with CAN has gradually been raised to 25 ml. THP tablets containing 225 mg of the commercial pharmaceutical were weighed, and the average weight was calculated. Then, the tablets were powdered and after well-filtering, THP was dissolved in 25ml.

**Apparatus**

Two apparatuses were used in this research to measure the amounts and absorbance of pharmaceuticals; they included:

1- The Biochemistry Analyzer, company: Geno Tek Inc., Generation: V4.8.3.1, Apparatus number: S/N: AES2K1KH01212.

2- A UV-visible spectrophotometer used to estimate the intensity of light transmitted through a sample by comparing it to a reference measurement of the incident light source, company: Apple, Code: 302372, PD: 303: Japan.

## Results

Multi concentrations of drugs (10, 30, 50, 70 and 100) ppm have been prepared and treated with different samples to measure the activity of CK-MB. The results of measuring CK-MB activity revealed increasing in the absorbance by increasing the concentration of drugs and the lowest readings are neglected (as shown in Table 1). Only

sample 1 concentrations are diluted with distilled water to read the absorbance so the final reading should multiply (×2).

**Table .1. The absorbance readings for the 5 concentrations of pure material CK-MB**

| Sample Number | Age and Sex | Pure material concentration (PPM) | Absorbance 1 Without the serum | Absorbance 2 With the serum |
|---|---|---|---|---|
| 1 | 43 yrs. Male | 10 | 0.149 | 0.147 |
| | | 30 | 0.143 | 0.151 |
| | | 50 | 0.142 | 0.15 |
| | | 70 | 0.127 | 0.138 |
| | | 100 | 0.147 | 0.153 |
| 2 | 41 yrs. Female | 10 | 0.459 | 0.564 |
| | | 30 | 0.252 | 0.25 |
| | | 50 | 0.23 | 0.224 |
| | | 70 | 0.234 | 0.241 |
| | | 100 | 0.493 | 0.543 |
| 3 | 40 yrs. Male | 10 | 0.56 | 0.548 |
| | | 30 | 0.236 | 0.227 |
| | | 50 | 0.221 | 0.218 |
| | | 70 | 0.33 | 0.305 |
| | | 100 | 0.345 | 0.397 |
| 4 | 45 yrs. Male | 10 | 0.416 | 0.422 |
| | | 30 | 0.644 | 0.649 |
| | | 50 | 0.076 | 0.095 |
| | | 70 | 0.187 | 0.201 |
| | | 100 | 0.043 | 0.037 |

The results of measuring of LDH activity revealed increasing in the absorbance with increasing of the concentration of drugs (Table.2). The lowest readings are neglected. A after finish the readings of the pure material the steps was repeated for tablet material (Guaifenesin and Theophylline) to obtain the absorbance's before and after adding reagents in the serum that contain drugs (table. 2 ,3 and 4 ) explaining the obtained results.

**Table .2: The absorbance readings for the 5 concentrations of pure material LDH**

| Sample Number | Age and Sex | Pure material concentration (PPM) | Absorbance 1 Without the serum | Absorbance 2 With the serum |
|---|---|---|---|---|
| 1 | 43 yrs. Male | 10 | 0.149 | 0.147 |
| | | 30 | 0.143 | 0.151 |
| | | 50 | 0.142 | 0.15 |
| | | 70 | 0.127 | 0.138 |
| | | 100 | 0.147 | 0.153 |
| 2 | 41 yrs. Female | 10 | 0.459 | 0.564 |
| | | 30 | 0.252 | 0.25 |
| | | 50 | 0.23 | 0.224 |
| | | 70 | 0.234 | 0.241 |

| | | | | |
|---|---|---|---|---|
| | | 100 | 0.493 | 0.543 |
| 3 | 40 yrs. Male | 10 | 0.56 | 0.548 |
| | | 30 | 0.236 | 0.227 |
| | | 50 | 0.221 | 0.218 |
| | | 70 | 0.33 | 0.305 |
| | | 100 | 0.345 | 0.397 |
| 4 | 45 yrs. Male | 10 | 0.416 | 0.422 |
| | | 30 | 0.644 | 0.649 |
| | | 50 | 0.076 | 0.095 |
| | | 70 | 0.187 | 0.201 |
| | | 100 | 0.043 | 0.037 |

## Table .3. The absorbance readings for the 5 concentrations of tablet material of CK-MB

| Sample Number | Age and Sex | Pure material concentration (PPM) | Absorbance 1 Without serum | Absorbance 2With serum |
|---|---|---|---|---|
| 1 | 43 yrs. Male | 10 | 0.105 | 0.116 |
| | | 30 | 0.224 | 0.218 |
| | | 50 | 0.2 | 0.201 |
| | | 70 | 0.176 | 0.186 |
| | | 100 | 0.25 | 0.235 |
| 2 | 41 yrs. Female | 10 | 0.48 | 0.129 |
| | | 30 | 0.162 | 0.17 |
| | | 50 | 0.214 | 0.242 |
| | | 70 | 0.224 | 0.264 |
| | | 100 | 0.159 | 0.18 |
| 3 | 40 yrs. Male | 10 | 0.167 | 0.171 |
| | | 30 | 0.864 | 1.461 |
| | | 50 | 0.106 | 0.109 |
| | | 70 | 0.176 | 0.169 |
| | | 100 | 0.124 | 0.143 |
| 4 | 45 yrs. Male | 10 | 0.136 | 0.135 |
| | | 30 | 0.123 | 0.122 |
| | | 50 | 0.27 | 0.872 |
| | | 70 | 0.725 | 0.659 |
| | | 100 | 0.289 | 0.276 |

## Table .4. The absorbance readings for the concentrations of tablet material ofLDH

| Sample Number | Age and SeX | Pure material concentration (PPM) | Absorbance 1 Without serum | Absorbance 2With serum |
|---|---|---|---|---|
| 1 | 43 yrs. Male | 10 | 1.307 | 1.304 |
| | | 30 | 1.494 | 1.487 |
| | | 50 | 1.18 | 1,159 |
| | | 70 | 1.522 | 1.572 |
| | | 100 | 1.505 | 1.49 |
| 2. | 41 yrs Female | 10 | 1.349 | 1.344 |
| | | 30 | 1.581 | 1.578 |
| | | 50 | 1.48 | 1.445 |
| | | 70 | 1.436 | 1.435 |
| | | 100 | 0.496 | 0.493 |
| 3 | 40 yrs. Male | 10 | 1.072 | 1.06 |
| | | 30 | 1.334 | 1.311 |
| | | 50 | 1.35 | 1.332 |
| | | 70 | 1.39 | 1.377 |

| | | | | |
|---|---|---|---|---|
| | | 100 | 1.276 | 1.257 |
| 4 | 45 yrs. Male | 10 | 1.344 | 1.332 |
| | | 30 | 1.419 | 1.402 |
| | | 50 | 1.18 | 1.166 |
| | | 70 | 1.412 | 1.404 |
| | | 100 | 1.262 | 1.256 |

**Second analysis by Biochemistry Analyzer:**

After analyzing the samples by biochemistry analyzer, the calculations done according to the following equations [11,12,13]:

$Activity = \Delta Absorbance \times 4500$ for CK-MB

$$Activation = \frac{Activity\ with\ drug}{Activity\ without\ drug} \times 100\%$$

Due to the high activity of samples 2 & 3, it has been used for the comparison of effectiveness after treatment with drugs. (Table. 5. 6) After treating sample 2 by drugs the results have been shown that when increasing the concentration, the activity of CK-MB increased as shown in (Figure .3). Theophylline and guaifenesin combination is used to treat or prevent the symptoms of asthma or to treat chronic bronchitis and emphysema. The results give good amount activated \with human blood (14) .

**Table .5. Bio chemical A CK-MB results**

| CK-MB | Guaifenesin and Theophylline | NormalValue | 20 | Factor | 4500 |
|---|---|---|---|---|---|

| **Sample No.** | **Pure Drugs Concentration** | **Activity** | **% Activation** | **Sample No.** | **Tablet Concentration** | **Activity** | **% Activation** |
|---|---|---|---|---|---|---|---|
| 2 | 10 | 13.50 | 67.500 | 2 | 10 | 72.00 | 360.000 |
| | 30 | 40.50 | 202.500 | | 30 | 94.50 | 472.500 |
| | 50 | 54.00 | 270.000 | | 50 | 112.50 | 562.500 |
| | 70 | 112.50 | 562.500 | | 70 | 126.30 | 631.500 |
| | 100 | 234.00 | 1170.000 | | 100 | 180.42 | 902.100 |

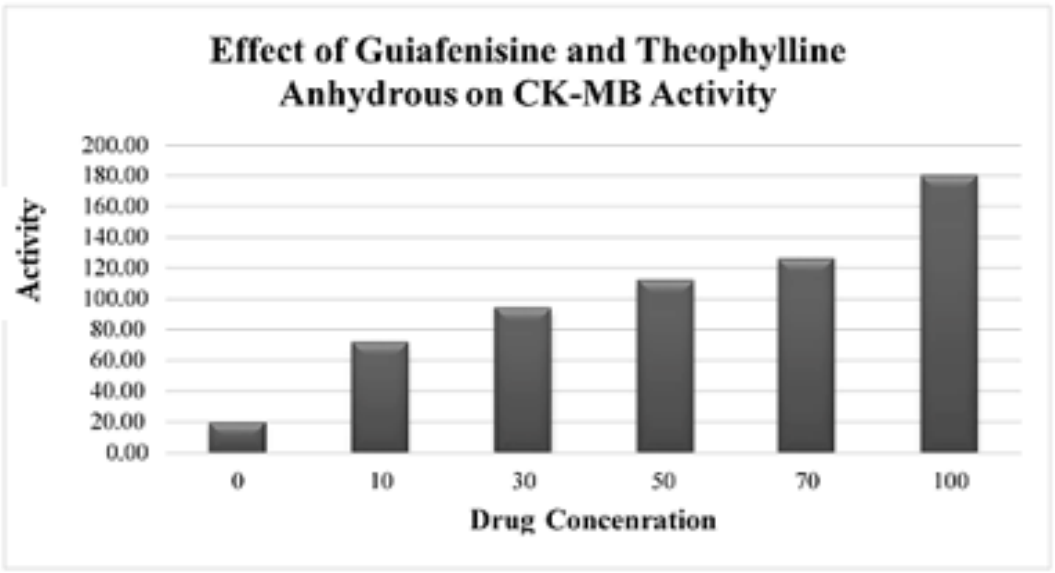


Figure .3.-Effect of Guaifenesin and Theophylline on CK-MB Activity

The results of this study also revealed a relation between the increasing of drug concentration and the decreasing in LDH level in sample 3 as shown in (Figure 4. Table .6.)

**Chemistry Analyzer LDH results**

$$activity = \Delta Absorbance \times 8095 \quad \text{for LDH}$$

$$activation = \frac{activity\ with\ drug}{activity\ without\ drug} \times 100\%$$

Table .6. Bio chemical LDH results

| LDH | Guiafenisine and Theophylline | NormalValue | 633 | Factor | 8095 |
|---|---|---|---|---|---|

| Sample No. | Pure Drugs Concenteration | Activity | % Inhibition | Sample No. | Tablet Concentration | Activity | % Inhibition |
|---|---|---|---|---|---|---|---|
| 3 | 10 | 250.54 | 60.420 | 3 | 10 | 250.54 | 60.420 |
| | 30 | 145.71 | 76.981 | | 30 | 145.71 | 76.981 |
| | 50 | 137.62 | 78.260 | | 50 | 137.62 | 78.260 |
| | 70 | 80.95 | 87.212 | | 70 | 80.95 | 87.212 |
| | 100 | 16.19 | 97.442 | | 100 | 16.19 | 97.442 |

Figure (4) explain the results of effected two drugs with LDH activated ,show plots the distribution of a numeric variable's values as a series of concentrations of drugs covers a range of numeric values data points with a value within the corresponding to LDH.

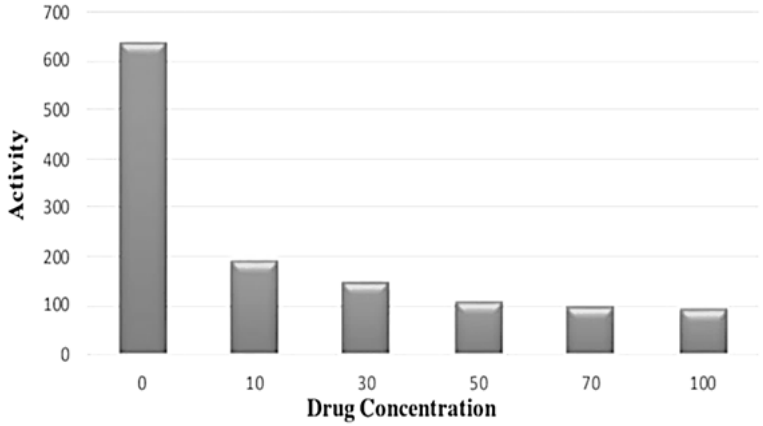


Figure .4. Effect of Guaifenesin and Theophylline on LDH Activity

The current study showed some limits. In this study, the 4th decade age groups in both genders have good oral health and gingival condition represented by low mean GI as illustrated clinically as decrease in redness, swelling, ulceration and bleeding from gingival tissue when examined and detected clinically this results were better seen in patients under Theophylline thismay be attributed to an improvement in maximum oxygen consumption which will have a positive outcomes on the cell metabolism and renewal and hence defensive mechanism (16-20).

## Discussion

Biochemical analyzer and UV-V is Spectrophotometric methods were developed for Guaifenesin and Theophylline which can be conveniently employed for routine analysis in pharmaceutical dosage forms and will eliminate unnecessary uninteresting sample preparations (21,22). The analysis conditions were optimized in order to provide a good performance of the assay and revealed same effect for Guaifenesin and Theophylline on blood samples in the treated body. The developed method was suitable, and very sensitive (23,24).

The results of this study also showed directly proportional between the concentration of the drug and the activity of enzyme CK-MB in sample 2 that collected from 41 years old female patient. Additionally, the increasing of the drug concentration lead to increasing in their activities of both pure and tablet drug and cause activation to CK-MB enzyme in the blood, which led to decreasing of the oral and gingival inflammation. However, inversely proportional were observed between the concentration of the drug and the activity of LDH enzyme in sample collected from patient 3 (40 years old male). These results were displaying a decreased in the activity of the drug with increasing of drug concentration in both form of the drug the pure and the tablet leading to inhibition of blood LDH enzyme. In conclusion, this study approved that the applied analytical

methods used in this study are useful for determination and estimation of these two drugs in blood of patients.

## DECLARATIONS

### Funding

The author stated that this work received no funding.

### Competing interests' statement

The author declare that they have no conflict of interest upon publication of this article from the third party.

### Ethics statement

I declare that the details in this application are the product of my own work and accurately reflect my research proposal. I have consulted an authoritative set of educational research ethics guidelines during the process of preparing this application. I understand that any substantive changes to the proposed research design may result in the requirement to submit another application for ethical approval to Ethical committee.

### Author contributions

The author provided the concepts, data analysis, and writing of the manuscript; worked with data collection and analysis; and revised the manuscript and analyzed the data.

### Acknowledgments

The author would like to extend their gratitude to the Scientific Center for Chemical Analysis for their support throughout this study

### References

1. Yi HC, Shi WS, Zhang YH, Zhu XZ, Yu Y, Wang XX, Dai Z, Lin Y. Comparison of electrolyte and glucose levels measured by a blood gas analyzer and an automated biochemistry analyzer among hospitalized patients. J Clin Lab Anal. 2020; 34(7):e23291. doi: 10.1002/jcla.23291

2. Jiuchuan Guo, Shulin Tian, Hong Xian, Lingyun Gao, Jinhua Xiang, Yongzhen Yang, Zou Yan , Yaochao Ning , Ke Liu and Jinhong Guo. Cyber–Physical Healthcare System with Blood Test Module on Broadcast Television Network for Remote Cardiovascular Disease (CVD) Management. IEEE Transactions on Industrial Informatics. 2021;17 (5):3663-3670. DOI: 10.1109/TII.2020.3010280

3. Shea EK, Hess RS. Validation of a flash glucose monitoring system in outpatient diabetic cats. J Vet Intern Med. 2021;35(4):1703-1712 https://doi.org/10.1111/jvim.16216.

4. Ganga NP. The Therapeutic Applications of Cough Medicines in Respiratory Diseases. Int J Pharm Phytopharmacol Res. 2021;11(3):6-10. https://doi.org/10.51847/DM6GuOGsgq

5. Ali Muthana S, Ali E. Karim, Maiser Z. Mohye, Azza M. Al-metwali, and Salah N. Ibrahim. "Simultaneous Determination of Guaifenesin, Codeine Phosphate, Phenylephrine Hydrochloride, and Sodium Benzoate in Syrup Pharmaceutical Form by RP-HPLC". Al-Kitab Journal for Pure Sciences.2023;7(2): 173–183. DOI: https://doi.org/10.32441/kjps.07.02.p13

6. Vemireddy Swapna, Kumaraswamy Gandla, Jyothsna B, and Bindu M. A validated RP-HPLC method for quantitative estimation of guaifenesin in bulk and pharmaceutical dosage forms. Research Journal of Pharmacy and Technology . 2023;160(1):111-114. DOI : 10.52711/0974-360X.2023.00020

7. Yingchun Duan, Anqing Wang, Yaping Ding, Li Li, Dingding Duan, Jiaxin Lin, Chenhong Yu, Jiayifan Liu and Yoshimine, Naoyuki. Fabrication of poly-sulfosalicylic acid film decorated pure carbon fiber as electrochemical sensing platform for detection of theophylline. Journal of Pharmaceutical and Biomedical Analysis. 2021;192:113663. https://doi.org/10.1016/j.jpba.2020.113663

8. Shivkanya Fuloria, Lau Tiew Wei, Sundram Karupiah, Vetriselvan Subramaniyan, Christina Gellknight, and Yuan Seng Wu, Saminathan Kayarohanam, Neeraj Kumar Fuloria , Development and validation of UV-visible method to determine gallic acid in hydroalcoholic extract of Erythrina fusca leaves ,International Journal of Research in Pharmaceutical Sciences.2020;11(4 ):6319-6326. https://doi.org/10.26452/ijrps.v11i4.3386

9. Vamshi Krishna Rapalli, Vedhant Kaul, Srividya Gorantla, Tejashree Waghule, Sunil Kumar Dubey, Murali Monohar Pandey, and Gautam Singhvi, UV Spectrophotometric method for characterization of curcumin loaded nanostructured lipid nanocarriers in simulated conditions: Method development, in-vitro and ex-vivo applications in topical delivery, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 5 January 2020;224:117392. https://doi.org/10.1016/j.saa.2019.117392

10. AL-Salman HNK, Ekhlas Qanber Jasim, Hussein H. Hussein , and Falah Hassan Shari, Theophylline Determination in PharmaceuticalsUsing a Novel High-performance Liquid Chromatographic Process, NeuroQuantology , July 2021;19 ( 7) :196-208 . doi: 10.14704/nq.2021.19.7.NQ21103

11. Wijnands WJ, Vree TB, Van Herwaarden CL. The influence of quinolone derivatives on theophylline clearance, British Journal of Clinical Pharmacology. 1986;22: 623-753. https://doi.org/10.1111/j.1365-2125.1986.tb02957

12. Kabaweh Mohamad and Sakur Amir Alhaj, Determination of some Bronchodilator and Expectorant Drugs (Guaifenesin and Theophylline) using HPLC Technique, Research Journal of Pharmacy and Technology.2019;12(4) (1915- 1918). DOI : 10.5958/0974-360X.2019.00320.2

13. Roy A Pleasants, Clinical Pharmacology of Oral Maintenance Therapies for Obstructive Lung Diseases, Respiratory Care. 2018;63 (6): 671-689. DOI: https://doi.org/10.4187/respcare.06068

14. Neven mikawy, Nancy Magdy, Marwa H. Mohamed and Amira M. El-Kosasy, High-Sensitive and Selective Spectroscopic Detection of Guaifenesin in Multiple Dosage Forms and Human Plasma , SSRN Support Center. 2023.

15. Jill A Ohar, James F Donohue, and Selwyn Spangenthal ,The Role of Guaifenesin in the Management of Chronic Mucus Hypersecretion Associated with Stable Chronic Bronchitis: A Comprehensive Review, Chronic Obstr. Pulm. Dis. 2019;23, 6(4):341-349. doi: 10.15326/jcopdf.6.4.2019.0139.

16. Arji Sravani Ratnam, Sravani Ratnam Arji , Sarma SRS Eranki, Anitha Kadimi , Prakash Nathanie, Kumar Sarella and Vinny Therissa Mangam,Development and validation of an HPLC method for the simultaneous estimation of salbutamol, theophylline and ambroxol in tablet dosage form. International Journal of Science and Research Archive. 2023;10(2):634-645. DOI: 10.30574/ijsra.2023.10.2.0983

17. Nurani Laela Hayu, Citra Ariani Edityaningrum, Irnawati Irnawati, Anggita Rosiana Putri, Anjar Windarsih, Any Guntarti ,and Abdul Rohman,Chemometrics-Assisted UV-Vis Spectrophotometry for Quality Control of Pharmaceuticals: A Review. Indonesian Journal of Chemistry.2023;23(2):542-567. https://doi.org/10.22146/ijc.74329

18. Gaddey Pridhvi Krishna, and Raja Sundararajan. ,Development of a Stability Indicating Method and Study of Forced Degradation Pathway of Choline Theophyllinate and Salbutamol by Liquid Chromatography–Mass Spectrometry, Chemistry Africa. 2024;7 (2), 777-798. https://doi.org/10.1007/s42250-023-00780-7

19. Ghanbari Mohammad Hossein, Zahra Norouzi, and Bastian JM Etzold. "Increasing sensitivity and selectivity for electrochemical sensing of uric acid and theophylline in real blood serum through multinary nanocomposites., Microchemical Journal. 2023;191:108836. https://doi.org/10.1016/j.microc.2023.108836

20. Chokkareddy Rajasekhar, Kilele Joan C, Kanchi Suvardhan, Redhi Gan G. Electrochemical Sensors for the Detection of Anti-asthma Drugs in Pharmaceutical and Biological Fluids: A Review, Current Analytical Chemistry.2023;19(3): 220-239. DOI: https://doi.org/10.2174/1573411019666230117152951

21. Aya A Mouhamed, Ahmed H Nadim, Nadia M Mostafa, and Basma M Eltanany. Application of smart chemometric models for spectra resolution and determination of challenging multi-action quaternary mixture: statistical comparison with greenness assessment. BMC chemistry. 2024;18(1) : 44. DOI https://doi.org/10.1186/s13065-024-01148-9

22. Mohammed M Al-Hinaai, Palanisamy Kannan, Jahangir Ahmad Rather, Rayees Ahmad Sheikh, and Mohammad Shahzad Samdani ,Ruthenium nanoparticles embedded poly (4-aminodiphenylamine) nanocomposites based determination of guaifenesin in

real samples, Journal of Saudi Chemical Society. 2024;28 (1):101776. https://doi.org/10.1016/j.jscs.2023.101776

23. Reem Hasan Obaydo, Hayam Mahmoud Lotfy, Sarah S Saleh, Dina Abbas Ahmed. Advanced Approaches in Green Univariate Spectrophotometric Methods. 2023. DOI: https://doi.org/10.1002/9781119889878.ch8. In book: Sustainable Approaches in Pharmaceutical SciencesPublisher: Wiley & Sons Ltd

24. Sharafi Effat, and Susan Sadeghi. A highly sensitive and ecofriendly assay platform for the simultaneous electrochemical determination of rifampicin and isoniazid in human serum and pharmaceutical formulations, New Journal of Chemistry .2023;47(1) :500-514. DOI: 10.1039/D2NJ04263K