Methods and objects of chemical analysis, 2024, Vol. 19, No. 3, 145–150

Aspirin Spectrophotometric Determination using Cloud Point Extraction and HPLC in Several Pharmaceuticals Sold in Iraqi Markets

Hanaa Abdullah Mohammed* and Safa Majeed Hameed**

Department of Chemistry, Faculty of Education for Women, University of Kufa, Al-Najaf 54001, Iraq;
*e-mail: rhanaaa.alghazali@student.uokufa.edu.iq; **e-mail: Safaa.alhassani@uokufa.edu.iq

Received: March 03, 2024; Accepted: May 15, 2024
DOI: 10.17721/moca.2024.145-150
Copyright © 2024 Hanaa Abdullah Mohammed. Open access article distributed under the Creative Commons Attribution License CC BY 4.0.

In this work, a sensitive tandem cloud-point extraction (CPE) method with a solvation system was employed to detect aspirin in various pharmaceuticals. Aspirin, in a dilute acidic solution, formed a complex with Zn(II) ions ([Zn(II)-aspirin] complex), which was then extracted using two different surfactant systems - non-ionic surfactant Triton X-100 and a mixture of surfactants (SDS-Tween 80). The extracted complex was subsequently determined using spectrophotometry at a wavelength of 293 nm for Triton X-100 and 231 nm for SDS-Tween 80. The parameters for extraction, including HCl and Zn(II) concentration, temperature, heating time, and solvent used to dissolve the cloud point layer, were thoroughly investigated. High-performance liquid chromatography (HPLC) was used as the standard method to compare the quantitative analysis of the cloud-point extraction-onium system.

Keywords: aspirin, SDS, Triton X-100, Zn(II), Tween 80

1. Introduction

Aspirin (AS), also known as 2-(acetyloxy) benzoic acid or acetylsalicylic acid, is a widely used and affordable drug available globally. It is a white powder with a melting point of 150 °C, has no odor, and has a slightly acidic taste. Aspirin is slightly soluble in water and soluble in ethanol [1]. The preparation process involves adding acetic anhydride to acetylsalicylic [2,3]. It belongs to the class of non-steroidal anti-inflammatory drugs, known for their ability to reduce inflammation, relieve pain, reduce fever, and decrease the likelihood of cardiovascular diseases [4]. Aspirin has been determined using a variety of analytical techniques, among them spectrophotometry [5-8], fluorescence spectroscopy [9], laser-induced photochemical [10], derivatives [11,12], the reverse-phase high-performance liquid chromatography method [13-15], titrimetry [16]), diazotization [17,18], and cloud point extraction [19].

Cloud point extraction (CPE) is a sensitive and selective preconcentration technique used for determining aspirin. The process involves dissolving the sample in an aqueous medium, adjusting the pH to ensure aspirin remains undissociated, and adding a nonionic surfactant like Triton X-114. In the CPE determination of aspirin, commonly used nonionic surfactants include Triton X-100, Triton X-114, Tween 20, Brij 35, and PONPE 7.5. These surfactants effectively form micelles that encapsulate aspirin molecules, enabling phase separation upon heating. Triton X-114 is particularly favored due to its low cloud point, which allows for efficient separation at moderate temperatures. The use of these surfactants enhances the sensitivity and selectivity of aspirin determination, making CPE a valuable technique in analytical chemistry.

In this work, we aim to present a novel cloud point extraction (CPE) technique with spectrophotometry for the instant determination and preconcentration of aspirin using Zn(II) as a chlorination to form ion-pair association complexes in a hydrochloric acid media. The approach proved to be efficient for detecting aspirin in a variety of pharmaceuticals. Furthermore, we used the HPLC methodology to compare the precision of the cloud point extraction method.

Experimental part

Chemicals and reagents

Aspirin (99.96%) and $ZnCl_2$ (Merck 99.8%) were performed from Merck and TritonX-100 (1%), SDS (1%) and Tween 80 (1%) from Alpha Chemika. All solvents (≥99 %, for HPLC grade) - acetonitrile, methanol, ethylene glycol, ethanol, 1-propanol, 2-propanol, 1-butanol, ethyl acetate, hexane, and 2-butanol were purchased from Merck.

Aspirin (100 μg/mL) were prepared by dissolving 0.01 g of the substance in a small amount of ethanol and then adjusting the volume to 100 mL with distilled water. To prepare the standard Zn(II) solution (1 mg/mL), 0.21 g of $ZnCl_2$ was dissolved in 100 mL of distilled water.

Mixture of surfactants (SDS-Tween 80) was prepared by mixing of 0.5 mL of solutions of SDS and Tween 80.

Instruments

A UV-Vis double beam spectrophotometer (Biochrom Libra S60, UK) was used for spectrophotometric studies and absorbance measurement. The thermostated water bath (Hamburg-90, England) maintained a constant temperature during the extraction experiments. Sykam S 600 Systems was used for HPLC measurements.

Preparation of samples

The process involved powdering and drying each medication at 105 °C for two hours. The medication was then cooled in a desiccator. Next, 50 mg of aspirin was placed into a 100 mL volumetric flask, and the volume was adjusted to the mark using ethanol and distilled water for cloud point extraction (CPE), and methanol for HPLC.

Extraction procedure

10 mL of aqueous solutions containing 100 μg/mL of zinc (II) and 100 μg/mL of aspirin in acidic media (HCl) were prepared. An appropriate amount (0.5mL) of 1% Triton X-100 or a combination of SDS-Tween 80 was included. The mixtures underwent heating in an electrostatic water bath at the designated time and temperature. Subsequently (95 °C), the cloud point layer was isolated from the aqueous solution and dissolved in 5 mL of ethanol. The absorbance was then measured at λmax, with ethanol serving as the blank solution.

HPLC parameters

Column - C18, flow rate - 1 mL/min, mobile phase: acetonitrile : methanol : 20mM sodium phosphate buffer at pH=3 (50:7:43), UV detection at 240 nm.

Results and Discussion

The spectrophotometric study reveals that Zn(II) onium species with aspirin, extracted into CPL, exhibit a wavelength of maximum absorbance at 293 nm when using Triton X-100 as a surfactant and at 231 nm when using SDS-Tween 80 as a mixture of surfactants (Fig. 1-2).

Optimization of CPE procedure

Amount of HCl concentration

In cloud point extraction (CPE) to determine aspirin quantity, hydrochloric acid (HCl) is used to adjust the pH to an acidic range, ensuring effective extraction of undissociated aspirin. Optimizing the amount of HCl based on preliminary experiments is crucial for efficient CPE performance in aspirin determination. The extraction procedure examines the impact of different concentrations of hydrochloric acid, ranging from 0.1 to 1.0 M, along with two surfactants: Triton X-100 or a combination of SDS-Tween80. The results are shown in Figure 3. The most favorable concentration of hydrochloric acid was determined to be 0.3 M in both Triton X-100 and SDS-Tween 80 surfactants.

Effect of temperature

Temperature is a key factor in creating controlled phase separation. Heating the solution above the cloud point temperature of the nonionic surfactant causes the surfactant to become more hydrophobic, leading to the separation of the surfactant-rich phase from the aqueous phase. The optimal temperature is critical for achieving maximum extraction efficiency and selectivity of the analyte, such as aspirin. The effect of temperature was studied in the range of 70°C to 95°C. The results are shown in Figure 4. The optimal temperature for the liquid ion exchange method with surface-active materials Triton X-100 and SDS-Tween 80 was found to be 90°C. This temperature significantly improved the aspirin extraction process. The effectiveness of this temperature can be explained by the requirement of a specific amount of energy for the formation of suitable aggregated micelles of surfactants, leading to a more efficient process.

Effect of heating time

Choosing the right heating time improves the extraction process by optimizing the cloud point layer (CPL). If the heating time is too long, it can reduce the effectiveness of the extraction and cause the micelles to disperse. When using a mixture of two surfactants, SDS-Tween80, each with its own critical

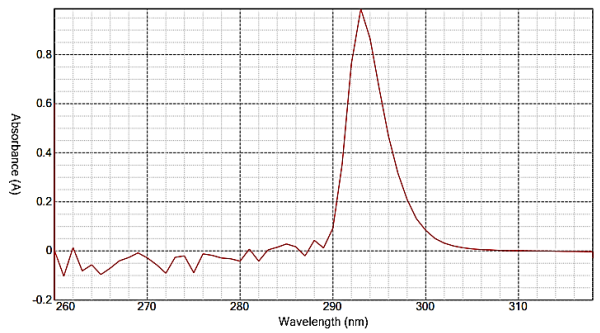

Figure 1. UV-Vis spectrum for onium species using Triton X-100 as non-ionic surfactant, λ_{max} = 293 nm.

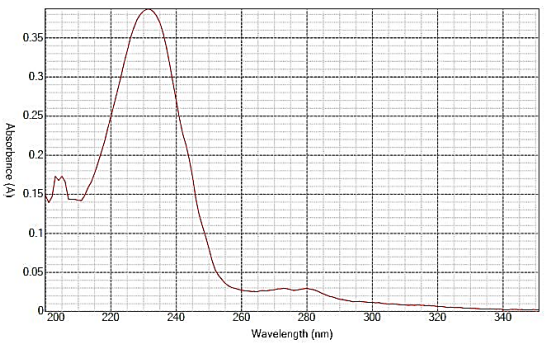

Figure 2. UV-Vis spectrum for onium species using SDS-Tween 80 as a mixture of non-ionic surfactant, λ_{max} = 231 nm.

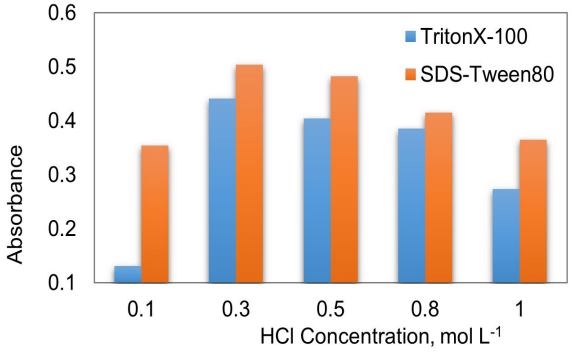

Figure 3. Effect of HCl concentration on Zn(II) onium species absorbance.

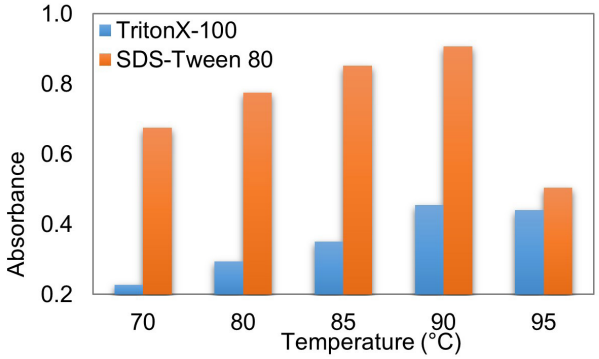

Figure 4. Effect of temperature on Zn(II) onium species absorbance.

micelle concentration (CMC), it takes longer to form a good cloud point layer [20]. In this study, aspirin was extracted using the extraction procedure at various heating periods (°C) with two surface-active substances: Triton X-100 or a mixture of SDS-Tween 80. The final results are shown in Figure 5. The test results indicate that Zn(II) with Triton X-100 can be detected after 15 minutes of heating, while with SDS-Tween 80 can be detected after 45 minutes.

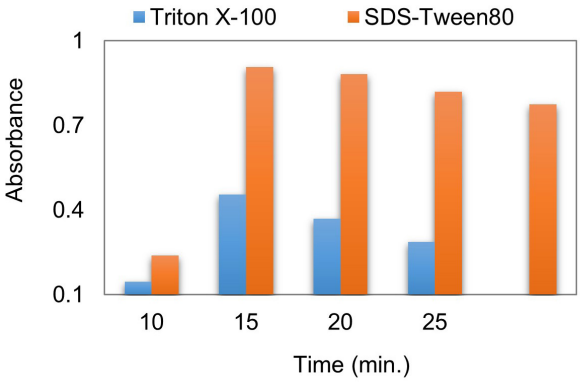

Figure 5. Effect of heating time on the absorbance of Zn (II) onium species.

Effect of Zinc (II) concentration

The concentration of Zinc (II) ions significantly affects the cloud point extraction (CPE) process for aspirin determination. High levels can interfere with extraction efficiency and require pH adjustments. Optimizing Zinc (II) concentration is crucial for accurate extraction. Preliminary experiments are essential to determine the maximum allowable concentration without affecting the CPE process. Based on the extraction procedure, the aspirin recovered from 10 mL of aqueous solutions comprises an increasing concentration of Zn(II) on two surfaces: TritonX-100 or SDS-Tween80. Figure 6 presents the results of the impact of Zn(II) ion concentration on the formation of onium species. The optimum Zn(II) ion concentration for extraction was determined to be 80 µg for the surfactants TritonX-100 and SDS-Tween80. Concentrations lower than this value are ineffective for achieving the best equilibrium, while concentrations higher than this value reduce extraction efficiency as per the mass action law [21, 22].

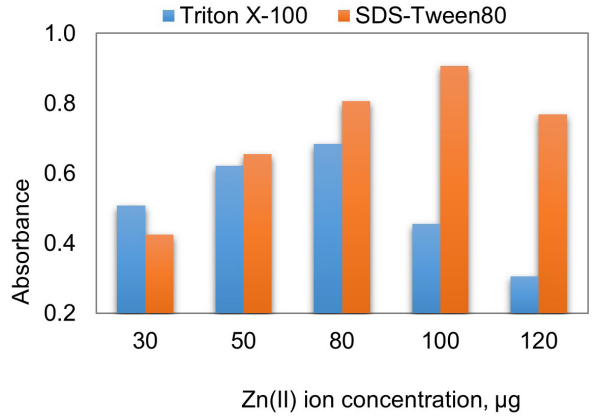

Figure 6. Effect of Zn(II) ion concentration on the onium species formation.

Role of solvents

The extraction procedure was used to separate aspirin from 10 mL of aqueous solutions under optimal conditions. The CPL was dissolved in a variety of organic solvents mentioned in Table 1. Gati and Szalay's [23] equation explains the effect of the dielectric constant (ε_r), which can be expressed as:

$$\Delta\tilde{V} = \left[(a-b)\left(n^2 - 2\frac{1}{n^2} + 1\right)\right] + b\left(\frac{D-1}{D+1}\right) \ldots\ldots\ldots (1)$$

The results are presented in Table 1.

Analytical parameters and calibration curve

The combined cloud point extraction with the onium approach allows for the spectrophotometric determination of aspirin with Zn(II) as a chloroanion complex. For the extraction procedure, the calibration curve was constructed under ideal conditions using TritonX-100 at λ_{max} = 293 nm. The calibration curve y = 0.0015x + 0.5163; R^2 = 0.9987 is linear in the studied range of 20 – 1000 ppm. Analytical parameters for the determination of aspirin are presented in Table 2.

Table 1. Maximum wavelength and dielectric constant of the organic solvents used to dissolve CPL.

Solvent	Triton X-100 λ_{max} (nm)	SDS-Tween80 λ_{max} (nm)	Dielectric constant (ε_r) [24]	(D-1) /(D+1)
Ethylene glycol	295	-	37.70	0.948
Methanol	293	-	33.00	0.941
Ethanol	293	-	24.30	0.921
1-Propanol	295	-	20.10	0.905
2-Propanol	294	-	18.30	0.896
1-Butanol	294	243	15.80	0.881
Ethyl acetate	295	-	6.02	0.715
Hexane	-	231	1.90	0.310
2-Butanol	295	-	1.75	0.273

Table 2. Analytical parameters of the method.

Parameter	Value
λ_{max} (nm)	293
Bear's law obey (ppm)	20 – 1000
Quantity Limit (μg mL^{-1})	25.140
Molar absorptivity (L $mol^{-1}cm^{-1}$)	0.263×10^3
Detection Limit (μg mL^{-1})	16.693

HPLC analysis

The pharmaceuticals were tested for aspirin content usinghigh-performance liquid chromatography (HPLC) and compared to the CPE-Onium method. Figure 7 displays the chromatogram of the samples and Table 3 outlines the aspirin levels in various drugs as determined by both methods.

Conclusion

The cloud point extraction/onium method was employed to determine the concentration of aspirin spectrophotometrically using a zinc(II) complex from an aqueous solution. This method has significantly improved UV-Vis detection. It is also the safest and most cost-effective method due to its lower equipment and running costs, requiring only a small amount of surfactant (0.5mL) and 80 μg of Zn(II). The analytical parameters clearly indicate that this approach can be a viable alternative method for determining aspirin in pharmaceuticals. For different drugs, the CPE-onium method seems to yield better results than HPLC.

Table 3. Aspirin amount in different drugs by two methods: CPE-Onium method and HPLC method.

Name	Company	Manufacture	Dose	Label claim taken (mg/g)	Measured concentration		% Recovery	
					CPE-Onium	HPLC	CPE-Onium	HPLC
ASPIRIN (Tablet)	BRISTOL Laboratories Ltd.0.	United Kingdom	75 mg/ Tablet	75	74.49	72.64	99.32	96.85
ASPEGIC (Instant Powder)	OPELLA Health France	France	1000 mg	1000	998.98	998.94	99.89	99.89
Dospin (Capsule)	Ajanta	India	75 mg	75	74.91	74.58	99.88	99.44

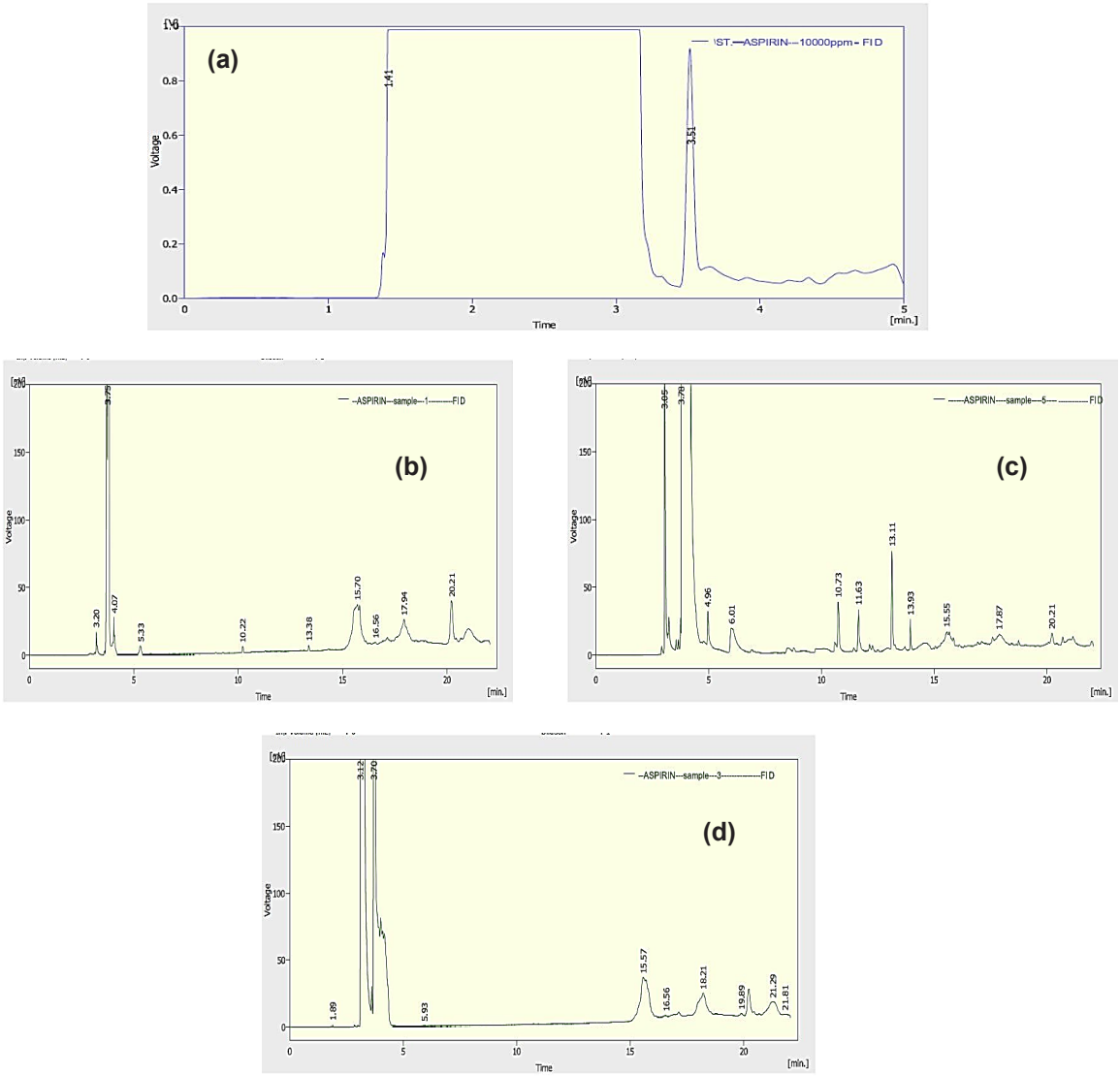

Figure 7. Chromatograms of (a) aspirin standard 1000 ppm, (b) Aspirin (tablet), (c) Dospin (capsule), (d) Aspegic (instant powder).

References

1. Senzana S., Gordana Z., Aleksandra N., Senzana B. and Salvinca M. “Quantitative analysis of acetylsalicylic acid in commercial pharmaceutical formulations and Human control serum using kinetic spectrophotometry, Acta chem solv, Vol. 55, pp.508-515, 2008

2. K. Schrör, Acetylsalicylic Acid. Germany: British Library Cataloguing-in-Publication Data, 2009.

3. C. S. Kwok and Y. K. Loke, “Critical overview on the Benefits and Harms of Aspirin,” Pharmaceuticals, vol. 3, pp. 1491-1506, 2010.

4. Cemel A., Ahmet S., “Rapid and simultaneous determination of Acetylsalicylic acid, paracetamol and their degradation and Toxic impurity product by HPLC in pharmaceutical dosage forms”, TUBITAK Journals medical science, Vol.38, pp.167-173, 2008.

5. Saraf S., Garg G., “Spectrophotometric determination of the aspirin and atenolol in combined dosage forms”, Indian Journal pharm. Edu. Res, Vol.42, pp.70-74, 2008.

6. G. Motan and A. Pui, “Studies of different types of aspirin by spectrophotometric methods,” ACTA CHEMICA IASI, vol. 22, pp. 155-164, 2014.

7. A. M. El-Didamony, M. Z. Saad, and N. O. Saleem, “Spectrophotometric determination of some analgesic drugs in pharmaceutical formulations using N-bromosuccinimide as an oxidant,” Journal of the Association of Arab Universities for Basic and Applied Sciences, vol. 17, pp. 43-50, 2015/04/01/ 2015.

8. Abo-Gharam, A. H., El-Kafrawy, D. S., Abdel-Khalek, M. M., Mahrous, M. S., & Belal, T. S. (2020). Spectrophotometric and chromatographic methods for simultaneous determination of aspirin and omeprazole. *Analytical Chemistry Letters*, *10*(2), 240-262.

9. Elmansi, H., Mohamed, S. A. E. A., & Fathy, M. E. (2016). Simultaneous determination of metoclopramide and aspirin by spectrofluorimetric technique: application to pharmaceutical formulations

and human plasma. *Analytical Methods*, *8*(6), 1281-1292.

10. El-Zahry, M. R., Refaat, I. H., Mohamed, H. A., & Lendl, B. (2016). Sequential SERS determination of aspirin and vitamin C using in situ laser-induced photochemical silver substrate synthesis in a moving flow cell. *Analytical and bioanalytical chemistry*, *408*, 4733-4741.

11. Ali, N. W., Abdelwahab, N. S., Abdelkawy, M., & Emam, A. A. (2012). Validated spectrophotometric and spectrodensitometric methods for determination of a ternary mixture of analgesic drugs in different dosage forms. *Bulletin of Faculty of Pharmacy, Cairo University*, *50*(2), 99-109.

12. Abdelazim, A. H., & Ramzy, S. (2022). Application of different quantitative analytical techniques for estimation of aspirin and omeprazole in pharmaceutical preparation. BMC chemistry, 16(1), 60.

13. Tiwari, P. K., Jain, A., Dubey, B. K., Pandey, G. K., & Dhakad, S. (2019). Analytical method development and validation for the simultaneous estimation of aspirin, clopidogrel and rosuvastatin in pharmaceutical dosage form. *Journal of Drug Delivery and Therapeutics*, *9*(4-s), 432-438.

14. Ali, L. M., Salih, M. A. H., & Hayder, O. I. (2019). Determination of Acetyl Salicylic Acid in Aspirin tablets. Kurdistan Journal of Applied Research, 4(2), 151-157.

15. Lirato, F., Al-Bassam, A., & Al-Sabea, N. (2010). Determination of Aspirin tablets from different industrial drug companies available in Iraqi pharmaceutical market. iraq journal of market research and consumer protection, 2(4).

16. Anthony, O., Moh'd, H. S., Mataka, M. A., Juma, A., & Odalo, O. J. (2017). A Comparative Anaylsis of Aspirin from Various Analgesic Formulations Using Titrimetry, Spectroscopic and Hypenated Chromatographic Techniques. J Mol Imaging Dynamic, 7(134), 2.

17. Mohammed, S. S. (2013). Comparative analytical study for determination of acetylsalicylic acid in bulk and in pharmaceutical formulations. Al-Nahrain Journal of Science, 16(1), 1-10.

18. Salim, A.M. and Haseeb, YSZ. (2013). Spectrophotometric Determination of Sulfadiazine via Diazotization and Coupling Reaction-Application to Pharmaceutical Preparations. Raf. J. Sci., 24 (6):61- 73.

19. Mubder, N. S., Al-Tameemi, M., Mahmood, H., Salman, N. K., & Al-Neshmi, H. (2022). Micro spectrophotometric determination and cloud point extraction of aspirin with iron (III) in pure form and pharmaceutical drugs. Chem. Methodol, 6, 569-570.

20. Azooz, E. A., Moslim, J. R., Hameed, S. M., Jawad, S. K., & Al-Mulla, E. A. J. (2021). Aspirin in food samples for separation and micro determination of copper (II) using cloud point extraction/solvation method. Nano Biomed. Eng, 13(1), 62-71.

21. Safa Majeed Hameed, Marwa Sabah Abdulwahid. Extraction and Preconcentration of Cd (II) by using Crown Ether DB18C6. Research Journal of Pharmacy and Technology. 2022; 15(6):2451-4. doi: 10.52711/0974-360X.2022.00409.

22. Hussain, S. A., Hameed, S. M., Abed, A. S., & Mahdi, N. S. (2023). Separation, Pre-concentration, and Determination of Al(III) by Cloud Point Extraction as a Compact Method with Liquid Ion Exchange. Journal of Medicinal and Chemical Sciences, 6(3), 656-667. doi: 10.26655/JMCHEMSCI.2023.3.22.

23. Mutar, M. M., & Ali, H. M.]2021[: Preparation, Characterization and Analytical Studies of novel Azo dyes and Diazo dyes. In Journal of Physics: Conference Series (Vol. 2063, No. 1, p. 012023). IOP Publishing.

24. Wohlfarth, C. (2008). Static dielectric constants of pure liquids and binary liquid mixtures: supplement to IV/6 (Vol. 17). Springer Science & Business Media.