ISSN 0974-3618 (Print)
0974-360X (Online)

www.rjptonline.org

RESEARCH ARTICLE

New approach for determination of Phenylephrine HCl in Pure and Pharmaceutical Formulation using a various Microextraction Methods

Nagham K. Kareem[1], Mohammed Z. Thani[2], Khalid F.Al-Rawi[3]
[1]Department of Pharmaceutical Chemistry, College of Pharmacy, University of Anbar, Ramadi. Iraq.
[2]Department of Chemistry, College of Science, University of Mustansiriyah, Baghdad, Iraq.
[3]Department of Chemistry, College of Science, University of Anbar, Ramadi, Iraq.
*Corresponding Author E-mail: **nag19s3007@uoanbar.edu.iq**

ABSTRACT:
In this study, ion pair reactions used for evaluation of phenylephrine HCl the development of simple, accurate, low-cost, and fast extraction methods for the separation of phenylephrine HCl was described. The first method was used was the direct extraction that included the reaction PHE with Alizarin yellow reagent to produce yellow complex in the acidic medium (pH=4). The absorbance of this complex was 430nm. The concentration range (1-20µg.mL^{-1}), the Beers law was obeyed with correlation coefficient (R^2=0.997), limit of detection as (0.34µg.mL^{-1}), limit of quantification as (1.12µg.mL^{-1}) and molar absorptivity as (14459.9). The second method was dispersive liquid liquid microextraction (DLLME). This method was used to estimation PHE complex by utilizing UV-Vis spectrophotometer. The linearity of calibration curve above was the range between (0.5-13 µg.mL^{-1}), the correlation coefficient (R^2=0.996) and molar absorptivity was (12423.3), the detection limit (LOD) and quantification limit (LOQ) were (0.094µg.mL^{-1}) and (0.31µg.mL^{-1}), respectively. This process was successfully method to detect PHE in both pure and pharmaceutical formulations.

KEYWORDS: PhenylephrineHCl (PHE), Direct extraction, DLLME, Spectrophotometer, Pharmaceutical formulation.

1. INTRODUCTION:
Phenylephrine HCl (PHE), as known as [(R)-1-3-(hydroxyl phenyl)-2-(methyl amino) ethanol hydrochloride], $C_9H_{13}O_2N.HCl$[1]. PhenylephrineHCl drugs are white crystalline powder or light yellow salt[2]. It represents the type of drugs called sympathomimetics that directly affect the adrenergic receptor[3]. It behaves as a stimulator for alpha receptors. It is used widely as a decongestant for sinusitis and nasopharyngitis, non-specific and allergic conjunctivitis[4]. The drug PHE is found in vasopressor medicines as eyewashes, nasal decongestant, and syrup[5]. Many of methods have suggested to determination of PHE in pharmaceutical formulations[6–7] such as High-performance liquid chromatography (HPLC)[8-9], voltammetry[10], titmetry[11], fluorescence[12], flow injection[13], UV-Vis Spectrophotometry[14-15-16].

Received on 13.06.2021 Modified on 29.07.2021
Accepted on 28.08.2021
Research J. Pharm.and Tech 2022; 15(4):1648-1652.
DOI: 10.52711/0974-360X.2022.00275

The dispersive liquid liquid microextraction (DLLME) has many advantages in the estimation of pharmaceutical preparation like rapid, safety and low cost[17-18]. It has applied as one of the determination and pre-concentration methods in analytical chemistry[19-20]. In this work, the proposed procedure is based ion-pair reaction of PHE with alizarin yellow reagent in the acidic media, then assessment and pre-concentration the usage dispersive liquid liquid microextraction (DLLME). The aim of this research is to classify, investigate, and determine the best conditions for quantifying PHE drugs using two different methods. One of them is directs extraction method at λ_{max} 430nm and the second one is dispersive liquid liquid microextraction (DLLME) and then compare between the two methods.

2. MATERIALS AND METHODS:
2.1. Materials:
Double beam UV-Vis spectrophotometer was used for all spectral and absorption intensity measurements with corresponded 1cm quartz cells. All the pH measurements were carried out by a Metrohm 780

digital pH meter(Switzerland) with a combined glass electrode.An IKA genius 3 vortex mixer (Staufen, Germany) was employed in extraction procedure. A Hermle Z-300 centrifuge (Wehingen, Germany) was used to separate the organic from aqueous phase.

2.2. Methods:

The chemicals PhenylephrineHCl (PHE) from Sigma – Aldrich, hydrochloric acid from Scharla, alizarin yellow from sigma-Aldrich, acetic acid, chloroform, ethanol, methanol, carbon tetrachloride were purchased from BDH(England).Double distilled water was used throughout the experiments.A 500µg/mL stock solution of PHE was prepared by dissolving 0.05gm of PHE in10 mL of 0.1N HCl and making up to 100ml with double distilled water in a volumetric flask. Acetate buffer solution was prepared by the addition of (9mL) of 0.1M sodium acetate to (41mL) of acetic acid 0.1M to adjust the pH at 4.0 and the mixture is brought up to 100ml with double distill water.

2.3. Pharmaceutical preparations procedure:

Three concentration of PhenylephrineHCl (eye drops 10% w/v) (European)5, 10, 15µg.mL^{-1} were taken, they were treated in the same way as direct and DLLME extraction in pure drugs, and the absorbance was measured at a wavelength of 430nm.Phenylephrine Hydrochloride BP (Dolo-cold 10mg PEP). Tablets (India) 50 mg, are carefully weighed, the pills are milled an Dissolved in 0.1N HCl and the filtered it complete the volume up to in a 100ml volumetric flask. They were treated in the same way as direct and DLLME extraction in pure drugs, and the absorbance was measured at a wavelength of 430nm.

2.4. General procedure of direct extraction for PHE medications:

A 0.5mL standard PHE drug solution was moved to a 5mL volumetric flask stoppered tube containing 2mL alizarinyellow reagent solution, and 1.2mL of acetate buffer (PH=4). The volume was adjusted to 5mL with 0.1N Hydrochloric acid. Chloroform 5mL was added and mixed well for one minute using vortex. The drug-reagent ion pair complex was extracted in chloroform. The chloroform layer was separated out. The absorbance of the colored solution was scanned on spectrophotometer in the range of 300-700nm against reagent blank. The blank was prepared similarly in which volume of standard solution of amino drug was replaced by an equal volume of 0.1N Hydrochloric acid.

2.5. General procedure of dispersive liquid liquid microextraction (DLLME) for amino medications:

In a 15mL glass centrifuge tube, add 0.5mL of medication (20µg.mL^{-1}), 1mL of alizarin yellow reagent (20µg.mL^{-1}), and 0.8 mL of acetate buffer (PH=4), then complete the volume to 5mL with 0.1N HCl, and then complete to 10mL with distilled water. Volume of 400µL chloroform as an extraction solvent and ethanol 700µL as dispersive solvent were rapidly injected into the solution using micro syringe to produce the formation of a cloudy solution. The mixture was centrifuged at 2000rpm for 6 minutes. The yellow ion pair complex was obtained with a micro syringe, put in a 1cm Quartz cell, and the absorbance at 430nm was measured against a blank.

3. RESULTS AND DISCUSSION:

The ion-pair complex is formed when the cation of phenylephrine HCl (PH^+) binds to the anion of theAlizarinyellow reagent(A^-) to form an intense yellow-colored ion-pair complex (A^- _ PH+). The absorbance of the yellow complex can be measured by spectrophotometer analysis in pH 4 at 430nm against blank; the results were illustrated in the (figure 1).

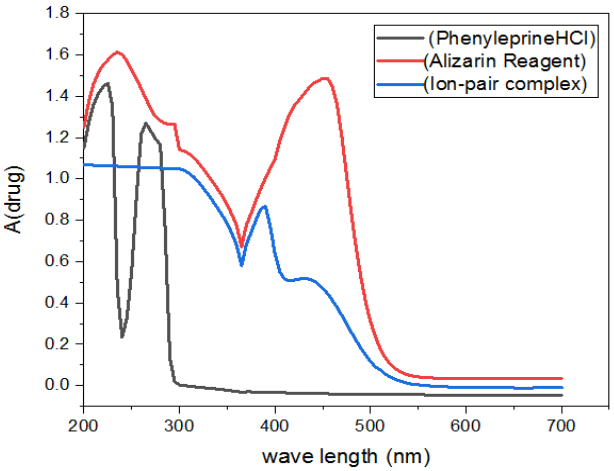

Figure. 1. Absorption Spectra of the Resulting complex.

3.1. Optimization of direct extraction:

The direct extraction combine with the UV-Vis. Spectrophotometer was used to select the suitable conditions for the complexity of phenylephrine HCl drug and a alizarin yellow reagent at wavelength 430nm, the most critical factor in this complex formation process is the acidic function (pH**),** type pH and volume of pH, reagent volume and type solvent. The range of pH for the phosphate buffer was used between (1-8), and it was noticed that the best acidity function for the complex formation was at pH = 4. Since the type of buffer has an effect on the formation of complexes, a variety of buffer solutions such as phosphate, acetate, and citrate buffer were used, and it was noticed that the acetate buffer gave the highest absorption value.

The different volumes of acetate were eximinated, and it was observed that a volume of 1.2mL had the highest absorption value at 430nm. This volume was considered the best for the complex formation between the phenylephrine HCl and an alizarin yellow reagent. The

volume of reagent needed for complex formation was determined to be 2 mL.

The influence of several solvents (chloroform, carbon tetrachloride, benzene, and hexane) on complex formation was also investigated; Chloroform was showed to be the optimum solvent for obtaining the best absorption. Two methods were used to denote the ratio of phenylephrine HCl and alizarin reagent, continuous variation and the molar ratio techniques, it was noticed that the ratio for both techniques was 1:1 (phenylephrine HCl drug: alizarin yellow reagent) (fig.2).

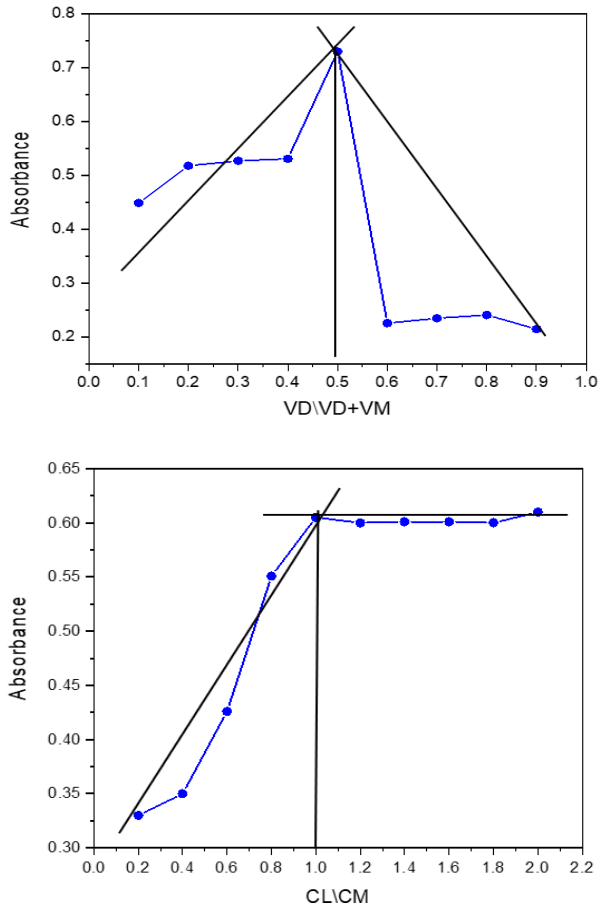

Fig.2: Continuous variation method and Mole-ratio method of Phenylephrine HCl

The interferences effect from several compounds such as starch, glucose, fructose, and others was investigated, and it was found that they had no influence on the extraction recovery, as shown in the Table.1.

Table.1: Extraction recovery with different interference compound

Compound	Recovery%
Maltose	99.4
Starch	97.8
Glucose	98.3
Lactose	96.6
Fructose	99.5
Glysine	96.6

3.2. Calibration curve:

After the identification of the optimal conditions for complex formation between phenylephrine HCl and an alizarin yellow reagent. The calibration curve was constructed using the absorbance against concentration of Phenylephrine HCl. The concentration range was ranges (1-20µg.mL^{-1}). The regression equation of calibration curve HCl is Y=0.071X+0.061 and R^2=0.997 of the linear calibration (Fig.3)

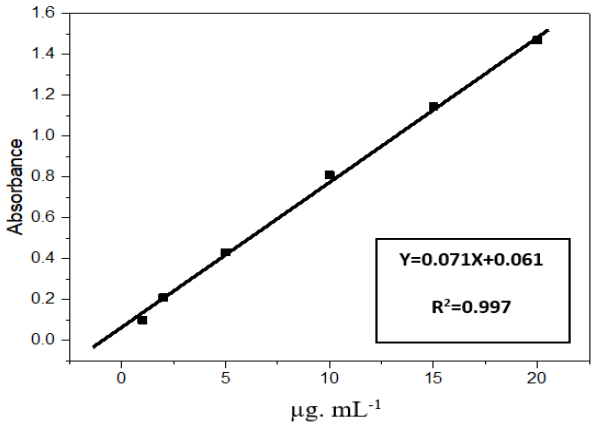

Figure. 3 : Standard calibration curve of complex formation

3.3. Optimization of dispersive liquid liquid Microextraction (DLLME):

At wavelength 430nm, the DLLME combine with a UV-Vis. spectrophotometer was used to select the best conditions for the complexity of phenylephrine HCl drug and an alizarin yellow reagent. The effect of the extraction solvent (chloroform, tetra chlorocarbon, and benzene), as well as the dispersed solvent (ethanol, methanol, acetone, and acetonitrile). was investigated, the result was showed that chloroform and ethanol were the best extraction and dispersion solvents for complex formation.

The acidity function, type of buffer solution, and Volume of buffer solution were also tested, the range of pH of phosphate buffer which used is between (1-8). It was also noticed that the best acidity function for the formation of the complex was at pH = 4. Since the type of buffer has an effect on the forming of complexes, a variety of buffer solutions (phosphate, acetate, and citrate) were used, and it was noticed that the acetate buffer gave the highest absorption value.

The different volumes of acetate were studied, and it was noticed that the volume of 0.8 ml had the highest absorption value at 430nm. This volume was considered the best for the complex formation between the phenylephrine HCl and an alizarin yellow reagent. The volume of Alizarin yellow reagent needed for complex formation was determined to be 1mL.

The best volumes for both the extraction and dispersion solvents were found to be 400µL and 700µL, respectively. The effect of speed and time in the centrifuge plays an important role in the formation of complexes. The best speed and extraction time were 6 minutes and 2000rpm.

The effect of several interferences such as starch, glucose, fructose, and others was investigated, and it was found that they had no influence on the estimated substance's absorbance value, as shown in the Table.2.

Table.2: Interference study

Compound	Recovery%
Maltose	96.8
Starch	95.3
Glucose	96
Lactose	97.8
Fructose	97.7
Glysin	99

3.4. Calibration curve:

After the identification of the optimal conditions for complex formation between phenylephrine HCl and an alizarin yellow reagent. The calibration curve was constructed using the absorbance against concentration of Phenylephrine HCl. The concentration range in was shown to be (0.5-13µg.mL^{-1}). The regression equation of phenylephrine HCl is Y=0.061X-0.032 and R^2=0.996 of the linear calibration (fig.4).

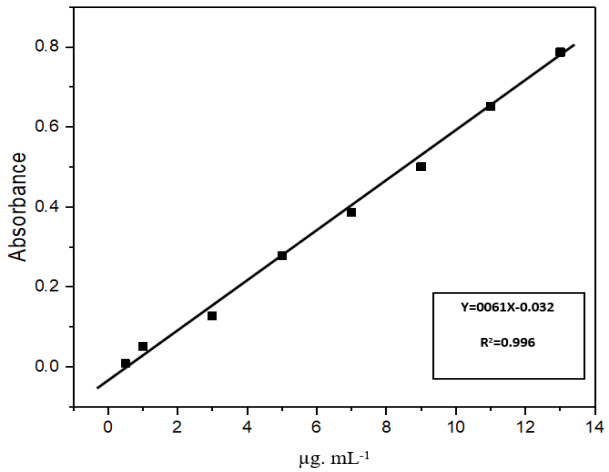

Figure. 4 : Standard calibration curve of complex formation(DLLME)

Table.3 Analytical parameter of direct extraction and DLLME method.

Parameters	Direct extraction	DLLME
λ_{max} nm	430	
Color	yellow	
Regression equation	Y=0.071X+ 0.061	Y=0.061X- 0.032
Linearty range(µg/mL^{-1})	1-20	1-13
Correlation Cofficient (R^2)	0.997	0.996
Ɛ(L.mol^{-1}.cm^{-1})	14459.9	12423.3
Sandell' ssensivity (µg. cm^{-2})	0.0141	0.0164
Slope (b)	0.071	0.061
Intercept(a)	0.061	0.032-
Limit of detection(µg/mL^{-1})	0.34	0.094
Limit of quantification(µg/mL^{-1})	1.12	0.31
C.L.for the slope($b\pm ts_b$) at 95%	0.071± 0.275	0.061±0.266
C.L.for the intercept($a\pm ts_a$) at 95%	0.061±2.96	0.032 ± 1.42
Standard error for regression line ($S_{y/x}$)	0.45	0.176
*C.L for Conc.X_1µg ml^{-1}at 95%	5.09±0.1241	2.8±0.0015
*C.L for Conc.$X_{2\mu g}$ ml^{-1}at 95%	9.82± 0.099	5.12±0.0025
*C.L for Conc.X_3µg ml^{-1}at 95%	14.04±0.174	6.85±0.0025
*Direct extraction (X_1=5, X_2=10, X_3=15) * DLLME (X_1=3, X_2=5, X_3=7)		

Table.4: Application of the proposed direct extraction and DLLME for the evaluation of phenylephrine HCl

drug	direct extraction					
	Conc. of drug mg.L^{-1}		Relative Error%	Recov. %	Average Recov%	RSD% (n=3)
	Taken	Found				
eye drops	5	5.2	-4	104	100	0.02
	10	10.2	-2	102		0.98
	15	14.1	6	94		0.015
Dolo-cold (tablet)	5	4.91	1.8	98.2	97.9	0.024
	10	9.35	6.5	93.5		0.021
	15	15.3	-2	102		0.04
DLLME						
eye drops	3	2.6	13.3	86.7	95.5	0.02
	5	5.2	-4	104		0.01
	7	6.7	4.3	95.7		0.04
Dolo-cold (tablet)	3	2.64	12	88	96.3	0.08
	5	5.1	-2	102		0.04
	7	6.92	1.1	98.8		0.01

Table.5: Comparison the values of Linearity, LOD and Recovery of the direct extraction and DLLME method with various methods reported in literature.

Method	Linearityµg/ml	LOD mg\L	Recovery	Ref.
Conductometric titration	8.0-50	2.5	100.113	11
Thin-layer chromatography	1.00 -10.00	0.30	98.70 ± 1.494	21-22
voltammetry	100–800	0.76	101.1 ± 0.3	23-24
Flow injection	0.03–8	20	—	25
UV-Vis Spectrophotometry	2 -30	0.200	99.90	26
UV-Vis Spectrophotometry	10-70	0.0557	100.27-100.31	27-28
UV-Vis Spectrophotometry	10-100	0.892	101.20	29
Direct extraction	1-20	0.34	97.88	Present work
DLLME	0.5-13	0.094	99.89	Present work

3.5. Accuracy and precision for direct extraction and DLLME method:

The calibration curve for phenylephrine HCl was used to determine the concentrations in the proposed procedure. In order to obtain accuracy in direct extraction and DLLME, the average drug was obtained, proving that this process was very accurate. Relative standard deviation was calculated and the recoveries predicted accuracy and reproducibility, by using three different concentrations. The results were appropriate for the proposed method.

3.6. Comparison with literature studies:

The DLLME method and the direct extraction method were compared to previously used phenylephrine HCl determination methods. In terms of RSD, Recovery, LOD, and other aspects, Table 5 demonstrates that the proposed method may be compared to and approved with previous procedures.

4. CONCLUSION:

Simple direct and DLLME extraction based on the extraction of an intense yellow bicolor ionic compound between phenylephrine hydrochloride and Alizarin yellow reagent, combined with UV –Vis. Spectrophotometry. The suggested direct extraction and DLLME method have been successfully used to extract and quantify phenylephrine hydrochloride in both pure and pharmaceutical preparations.

5. ACKNOWLEDGMENTS:

The authors express their gratitude to the Pharmaceutical Chemistry Branch of the College of Pharmacy as well as the Chemistry Department of the College of Science, University of Anbar, for providing all laboratory equipment.

6. REFERENCES:

1. Sasikala M, Priyanka P, Vinod Kumar T, Venkateshwarlu G. Spectrophotometric estimation of drugs using N-bromo succinamide and Indigo Caramine couple. Orient J Chem. 2016; 32(1):617-625. doi:10.13005/ojc/320170
2. Yagmur S, Ture M, Saglikoglu G, Sadikoglu M, Yilmaz S. The Quantitative Detection of Phenylephrine in Pharmaceutical Preparations and Spiked Human Urine by Voltammetry. Russ J Electrochem. 2018;54(10):741-746. doi:10.1134/S1023193518100063
3. Kalyankar TM, Wadher SJ, Bodhankar MR, Sayed MF. Stability Indicating Simultaneous Estimation of Phenylephrine HCl and Bromhexine HCl in Combined tablet dosage form by UV-Spectrophotometer. Res J Pharm Technol. 2021; 14(6):3128-3132.
4. Humeidy I. Determination of Trifluoperazine hydrochloride in pharmaceutical preparations using Spectrophotometric method. J Garmian Univ. 2017; 4(ICBS Conference):463-473. doi:10.24271/garmian.156
5. Srividya K, Balasubramanian N. Indirect spectrophotometric determination of thiamine in pharmaceutical preparations. Chem Pharm Bull. 1997; 45(12):2100-2103. doi:10.1248/cpb.45.2100
6. T. M. K, Wadher SJ, Bodhankar MR, Sayed MF. Manoranjan Sabat, Sharada Nalla, Venkateswarlu Goli, Sravan Prasad Macherla, Praveena Kumari Matta, Madhu Chandaka S. A New Analytical Method Development and Validation for Estimation of Ciprofloxacin in Bulk and Pharmaceutical Dosage Form. Asian J. Phar. Res J Pharm Technol. 2021; 14(June):3128-3132. doi:10.52711/0974-360x.2021.00545
7. Sabat M, Nalla S, Goli V, Macherla SP, Matta PK, Chandaka SM. A new analytical method development and validation for estimation of Ciprofloxacin in bulk and pharmaceutical dosage form. Asian J Pharm Anal. 2012; 2(4):116-117.
8. Mirzajani R, Kardani F. Fabrication of ciprofloxacin molecular imprinted polymer coating on a stainless steel wire as a selective solid-phase microextraction fiber for sensitive determination of fluoroquinolones in biological fluids and tablet formulation using HPLC-UV detection. J Pharm Biomed Anal. 2016; 122:98-109. doi:10.1016/j.jpba.2016.01.046
9. Chougule DD, Naikwade NS. Development and validation of high performance liquid chromatographic method for estimation of deflazacort in pharmaceutical formulation. Asian J Res Chem. 2011; 4(1):140-142.
10. Kalambate PK, Srivastava AK. Simultaneous voltammetric determination of paracetamol, cetirizine and phenylephrine using a multiwalled carbon nanotube-platinum nanoparticles nanocomposite modified carbon paste electrode. Sensors Actuators B Chem. 2016; 233:237-248.
11. H. Hasan S, S. Othman N, M. Surchi K. Determination of Phenylephrine-HCl Using Conductometric Titration Method. Curr Anal Chem. 2015; 12(4):330-334. doi:10.2174/1573412912666151126205443
12. Bahrami G, Mohammadi B, Mirzaeei S, Kiani A. Determination of atorvastatin in human serum by reversed-phase high-performance liquid chromatography with UV detection. J Chromatogr B Anal Technol Biomed Life Sci. 2005; 826(1-2):41-45. doi:10.1016/j.jchromb.2005.08.008
13. Metochlopramide U, Reagent C. Batch and Flow-Injection Spectrophotometric Determination of Methylyldopa. 2013; (March).
14. Felipe MAA. Offline machine learning-based concurrent and rapid determination of acetaminophen, dextromethorphan, guaifenesin, and phenylephrine using UV-vis spectroscopy. Published online 2021.
15. Alteemi HS, Kadim KH. Colorimetric Determination of Phenylephrine hydrochloride Drug by Diazotization Reaction. In: Journal of Physics: Conference Series. Vol 1664. IOP Publishing; 2020:12097.
16. Kadam T V, Darekar AB, Gondkar SB, Saudagar RB. Development and validation of spectrophotometric method for determination of azelaic acid. Asian J Res Pharm Sci. 2015; 5(2):83-85.
17. Xue L, Chen L, Dong J, Cai L, Wang Y, Chen X. Dispersive liquid-liquid microextraction coupled with surface enhanced Raman scattering for the rapid detection of sodium benzoate. Talanta. 2020; 208(September 2019):120360. doi:10.1016/j.talanta.2019.120360
18. Mashayekhi HA, Abroomand-Azar P, Saber-Tehrani M, Husain SW. Rapid determination of carbamazepine in human urine, plasma samples and water using DLLME followed by RP-LC. Chromatographia. 2010; 71(5-6):517-521. doi:10.1365/s10337-009-1456-6
19. Kraševec I, Prosen H. Development of a dispersive liquid-liquid microextraction followed by LC-MS/MS for determination of benzotriazoles in environmental waters. Acta Chim Slov. 2019;66(1):247-254. doi:10.17344/acsi.2018.4914
20. Quigley A, Cummins W, Connolly D. Dispersive liquid-liquid microextraction in the analysis of milk and dairy products: A review. J Chem. 2016; 2016. doi:10.1155/2016/4040165
21. Hegazy M, Al-ghobashy M, Eltanany B. Purity Indicating TLC Method for Quantitative Determination of Phenylephrine and Dimethindine Maleate in Presence of Dimethindine Maleate Impurity: 2-ethyl pyridine in Nasal Gel. J Pharm Res. 2016; 1(1). doi:10.33140/jpr/01/01/00004
22. Raju GVH, Ganapathy S, Sankar DG, Naidu PY. RP-HPLC Determination of Levetiracetam in Bulk and Pharmaceutical Formulation. Asian J Res Chem. 2009; 2(3):253-257.
23. Pourghobadi Z, Niazi A. Voltammetric study and determination of phenylephrine hydrochloride at INP-nafion-modified CPE sensor employing differential pulse voltammetry. Orient J Chem. 2014;30(1):219-227. doi:10.13005/ojc/300126
24. Sharma JB, Bhatt S, Saini V, Kumar M. Development and Validation of UV-Visible Spectrophotometric method for the Estimation of Curcumin and Tetrahydrocurcumin in Simulated Intestinal Fluid. Res J Pharm Technol. 2021; 14(6):2971-2975.
25. Al-Abachi MQ, Subhi S. Flow injection-Spectrophotometric Determination of Phenylephrine Hydrochloride and Amoxicillin Trihydrate in Pharmaceutical Preparations. J Al-Nahrain Univ Sci. 2013;16(1):42-52. doi:10.22401/jnus.16.1.07
26. Wadher SJ, Kalyankar TM, Panchal PP. Development and validation of simultaneous estimation of chlorpheniramine maleate and phenylephrine hydrochloride in bulk and capsule dosage form by ultra-violet spectrophotometry. Int J ChemTech Res. 2013; 5(5):2410-2419.
27. Anandakumar K, Veerasundari P. Simultaneous Estimation of Paracetamol, Ambroxol Hydrochloride, Levocetirizine Dihydrochloride, and Phenylephrine Hydrochloride in Combined Tablet Formulation by First-Order Derivative Spectrophotometry. ISRN Spectrosc. 2014; 2014:1-8. doi:10.1155/2014/248960
28. Balloul G, Boyko NN, Zhilyakova ET, Doba S. Development and validation of an analytical method for the determination of ofloxacin and benzyl alcohol in a pharmaceutical mixture. Res J Pharm Technol. 2021; 14(4):2133-2138. doi:10.52711/0974-360X.2021.00378
29. Chudiwal SS, Dehghan MHG. Development and validation of spectrophotometric method for budesonide estimation in nasal spray formulations. Indian Drugs. 2016; 53(7):42-45.