Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(11):68-74

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Determination of pipazethate hydrochloride, fenoterol hydrobromide and clopidogrel hydrogen sulphate using citrate-capped gold nanoparticles

M. M. Ayad, H. E. Abdellatef, M. M. Hosny and Y. A. Sharaf*

Analytical Chemistry Department, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt

---

ABSTRACT

Development of new and highly sensitive colorimetric method for the determination of pipazethate hydrochloride, fenoterol hydrobromide and clopidogrel hydrogen sulphate based on aggregation of the citrate capped gold nanoparticles (Au NPs). Citrate-capped gold nanoparticles were prepared by chemical reduction of HAuCl4 using citrate as the reducing agent and stabilizer. The synthesized nanoparticles showed an intense localized surface plasmon resonance absorption band at 520nm. Uniformly distributed spherical gold nanoparticles with average particle diameter size of 14.20±2.53 nm were verified through Transmission Electron Microscope (TEM). Addition of the cited drugs to the Au NPs solution results in aggregation of the Au NPs. As a result of aggregation, the plasmon band at around 520 nm disappears with the formation of a new red shifted band at 647- 674nm. The calibration curves were linear with concentrations of 0.4-0.8, 0.8-2 and 1.6-9.6 µg/ml for pipazethate hydrochloride, fenoterol hydrobromide and clopidogrel hydrogen sulphate, respectively. The detection limits were 0.100, 0.061 and 0.259 µg ml^{-1} for pipazethate hydrochloride, fenoterol hydrobromide and clopidogrel hydrogen sulphate, respectively. The method was applied successfully to the determination of the cited drugs in pure form and pharmaceutical dosage forms.

Keywords: Pipazethate hydrochloride; fenoterol hydrobromide; clopidogrel hydrogen sulphate; citrate- capped gold nanoparticles

---

INTRODUCTION

Pipazethate hydrochloride [2-(2-piperidinoethoxy) ethyl 10H-pyrido [3,2-b] benzothiadiazine-10-carboxylate hydrochloride][1] is a centrally acting cough suppressant which also has some peripheral actions in non-productive cough [1]. Different techniques were reported for the determination of pipazethate hydrochloride including: spectrophotometry [2 , 3], electrochemical [4] and chromatographic [5- 7] methods.

Fenoterol hydrobromide [(1RS)-1-(3,5-dihydoxyphenyl)-2-[[(1RS)-2-(4-hydroxyphenyl)-1-methylethyl] amino] ethanol hydrobromide.][8] is direct-acting sympathomimetic with beta-adrenoceptor stimulant activity largely selective for beta2 receptors (a beta2 agonist). It is used as a bronchodilator in the management of reversible airways obstruction, as occurs in asthma and in some patients with chronic obstructive pulmonary disease [1]. Various analytical methods have been applied for the determination of fenoterol hydrobromide in raw material, pharmaceuticals and biological fluids. These methods include chromatography [9, 10], voltammetry [11], electrophoresis[12 ,13], spectrophotometry[14] and spectroflourimetry[15].

Clopidogrel hydrogen sulphate [Methyl (S)-2-chlorophenyl(4,5,6,7-tetrahydrothieno[3,2-c]pyridin-5-yl)acetate bisulphate; Methyl (+)-(S)-α(o-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-acetate hydrogen sulphate] [8] is a thienopyridine antiplatelet drug used in thromboembolic disorders. It is an analogue of ticlopidine acts by inhibiting adenosine diphosphate-mediated platelet aggregation. It is given prophylactic ally as an alternative to aspirin in patients with atherosclerosis who are at risk of thromboembolic disorders such as myocardial infarction,

peripheral arterial disease, and stroke. It is also used with aspirin in patients with unstable angina, as an adjunct to medical or interventional management. Its role in coronary stenting is discussed under Reperfusion and Revascularisation Procedures [1]. Different methods were reported for determination of clopidogrel hydrogen sulphate including spectrophotometry [16, 17], liquid chromatography [18- 20] and thin layer chromatography[21].

Nanoparticles made of silver and gold have been the focus of research for many decades as a result of their intriguing optical properties [22]. When gold nanoparticles dispersed in liquid media, these nanoparticles exhibit a strong UV–visible extinction band at 520 nm that is not present in the spectrum of the bulk metal. Recently gold nanoparticles have been developed for sensitive and selective detection of urinary adenosine [23], Phenothiazine Drugs [24], Malathion, Fenthion, Methidathion [25], cysteamine [26], glutathione and cysteine [27].

Herein, we have developed a simple and highly sensitive spectrophotometric method for determination of pipazethate hydrochloride, fenoterol hydrobromide and clopidogrel hydrogen sulphate based on the aggregation of citrate capped gold nanoparticles (Au NPs), through employing the strong affinity characteristics of the cited drugs towards the surface of the Au NPs.

EXPERIMENTAL SECTION

2.1. Instrumentation

A Shimatzu UV and visible recording spectrophotometer (UV 260) with matched 10-20 quartz cell was employed for all absorbance measurements.

A JEOL-1010 Transmission Electron Microscope at 80 KV, Japan was employed for Transmission Electron Microscopy (TEM) examination at Regional Center for Mycology and Biotechnology (RCMB) at Al-Azhar University.

2.2. Materials and reagents

Chemicals used were of the highest purity

1-Pipazethate hydrochloride (obtained from Egyptian International Pharmaceutical Industries Company (EPICO))
2-Fenoterol hydrobromide (obtained from Sigma Pharmaceutical Industries)
3-Clopidogrel hydrogen sulphate (obtained from Sigma Pharmaceutical Industries)
4-Chloroauric acid ($HAuCl_4$)
5-Acetate buffer pH5: Dissolve 13.6 g of sodium acetate and 6 ml of glacial acetic acid in sufficient water to produce 1000 ml [8]

2.2.1. Pharmaceutical preparations

1- Selgon® tablets containing 20mg Pipazethate hydrochloride per tablet (obtained from Egyptian International Pharmaceutical Industries Company (EPICO))
2- Selgon® Drops containing 40 mg Pipazethate hydrochloride per ml solution (obtained from Egyptian International Pharmaceutical Industries Company (EPICO))
3- Pronotrol® Syrup containing 2.5 mg fenoterol HBr per 5ml (obtained from Sigma Pharmaceutical Industries)
4- Sigmagrel® tablets containing 75 mg clopidogrel hydrogen sulphate per tablet (obtained from Sigma Pharmaceutical Industries)

2.2.2..Standard solutions

Solutions of 100 µg ml^{-1} of pipazethate hydrochloride, fenoterol hydrobromide and clopidogrel hydrogen sulphate were prepared by dissolving 10 mg of the pure drug in bidistilled water then further dilution to 5,10 and 40 µg ml^{-1} for pipazethate hydrochloride, fenoterol hydrobromide and clopidogrel hydrogen sulphate respectively.

2.3. General procedure

2.3.1. Procedure for preparation of citrate-capped gold nanoparticles

Au NPs were prepared by sodium citrate reduction method [24, 27]. To a 150-mL beaker, 2.0 ml of 1% $HAuCl_4$ and about 100 ml of water were added and the solution was heated up to 95°C. 5.0 ml of 1% sodium citrate solution was added drop by drop while the solution is vigorously stirred. The solution was kept at 95°C for 10 min. When the color of solution changed to bright red, the solution was allowed to cool to room temperature and transferred into a 100 ml volumetric flask, diluted to the mark with water and mixed completely. The average size of the prepared Au NPs is about 14.20±2.53 nm, which is estimated from TEM image (Figure 1,2).

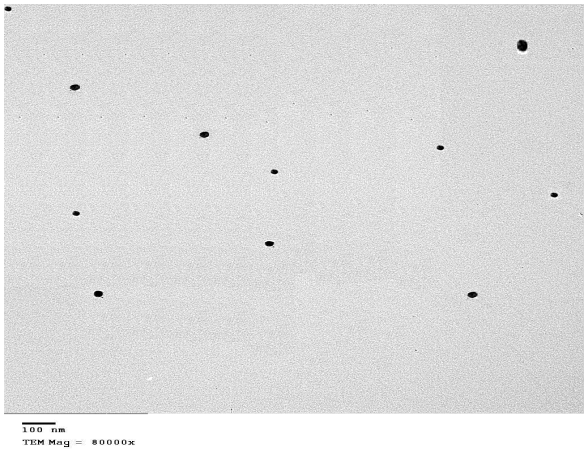

Figure 1 TEM microscope of the citrate-capped gold nanoparticles (Au NPs)

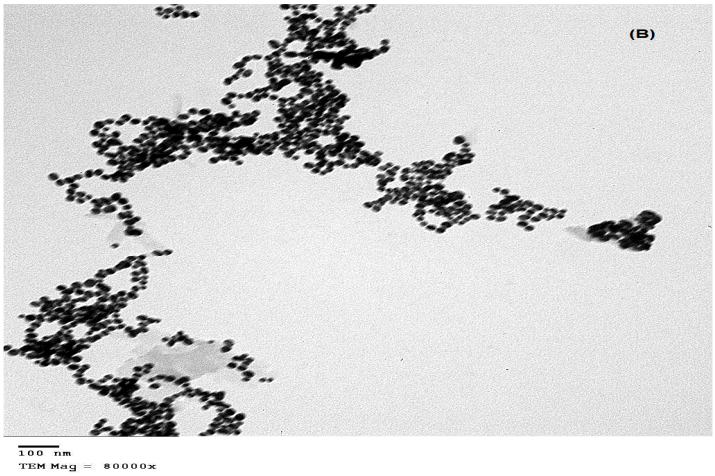

Figure 2 TEM microscope of aggregated gold nanoparticles (Au NPs) in the presence of pipazethate hydrochloride

2.3.2. Procedure for determination of pipazethate hydrochloride, fenoterol hydrobromide and clopidogrel hydrogen sulphate

In 5 ml volumetric flask, aliquots of the cited drugs were placed then appropriate volumes of acetate buffer pH5 and Au NPs solution were added, completed to 5ml with bidistilled water, and let to stand at room temperature for appropriate times. Absorbances were measured at suitable λ_{max} against reagent blank treated similarily. (Table 1)

Table 1 Analytical parameters for determination of pipazethate hydrochloride, fenoterol hydrobromide and clopidogrel hydrogen sulphate using citrate-capped gold nanoparticles (Au NPs)

Parameter	*Pipazethate hydrochloride*	*Fenoterol hydrobromide*	*Clopidogrel hydrogen sulphate*
λ_{max} (nm)	659	674	647
Volume of citrate-capped gold nanoparticles	3ml	3 ml	3 ml
Volume of buffer (pH 5)	0.25 ml	0.25ml	—
Temperature	25ºC	25ºC	25ºC
Time of reaction	15 min.	40 min.	50 min.
Aliquots taken (µg)	1.75 – 3.25	1.0 – 8.0	4.0 – 40.0

2.3.3. Assay of pharmaceutical preparations

A- Assay of tablets

Ten tablets were weighed, pulverized into fine powder, specific quantity of powdered drugs equivalent to 10 mg pure drug were dissolved in distilled water, solutions were filtered and diluted to 100ml with distilled water then further dilution to 5 and 40 µg ml^{-1} for pipazethate hydrochloride and clopidogrel hydrogen sulphate, respectively. Procedures were completed as in general procedures.

B- Assay of drops
Specific volume of drops solutions equivalent to 10 mg pure drug were placed in 100 ml volumetric flask and diluted to 100ml with distilled water then further dilution to 5 µg ml^{-1} for pipazethate hydrochloride. Procedure was completed as in general procedures.

C- Assay of syrup
Specific volume of syrup equivalent to 10 mg pure drug were placed in 100 ml volumetric flask and diluted to 100ml with distilled water then further dilution to 10 µg ml^{-1} for fenoterol hydrobromide. Procedure was completed as in general procedures.

RESULTS AND DISCUSSION

Nanoparticles made of silver and gold have been the focus of research for many decades due to their optical properties. The citrate-capped gold nanoparticles (Au NPs) were synthesized by chemical reduction using $HAuCl_4$ as the precursor salt and citrate as the reducing agent [28]. The pH value of the synthesized citrate-capped gold nanoparticles was measured to be around 6.0. UV-visible spectrum of citrate-capped gold nanoparticles (Au NPs) solution exhibits a well known absorption maximum at 520 nm as a result of localized surface plasmon resonance. Upon addition of the cited drugs, the color of Au NPs solution gradually changes from red to purple and accordingly the absorption band shifted to longer wavelengths. A new absorption peak appears at 659, 674 and 647 nm when pipazethate hydrochloride, fenoterol hydrobromide and clopidogrel hydrogen sulphate are mixed with Au NPs, respectively (Figure 3). This band can be attributed to displacement of the citrate group shell with the cited drugs leading to aggregation of Au NPs.

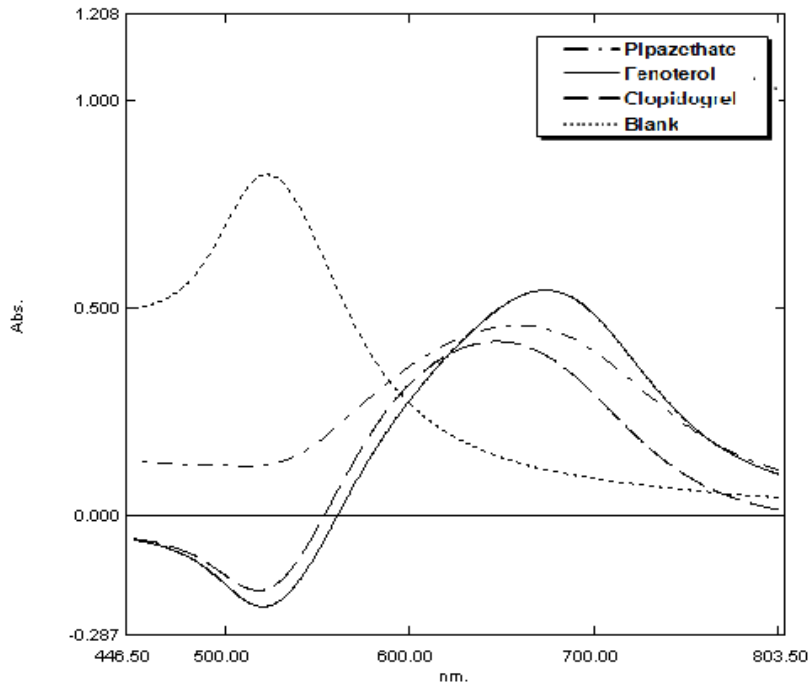

Figure 3 Absorbance spectra of the reaction between Au NPs and 0.5 µg ml^{-1} pipazethate hydrochloride, 0.8 µg ml^{-1} fenoterol hydrobromide, 3.2 µg ml^{-1} clopidogrel hydrogen sulphate

Optimum conditions affecting the reaction were studied

3.1.1. Effect of buffer pH
The pH of the solution plays an important role in the interaction between Au NPs and studied drug. Thus the influence of solution pH on absorbance was studied over the pH range of 2.0 - 8. Different buffer solutions (acetate buffer, phosphate buffer, chloride buffer and borate buffer) were tried. It was found that 0.25 ml of acetate buffer pH 5 was selected for fenoterol hydrobromide and clopidogrel hydrogen sulphate, while no buffer is required for pipazethate hydrochloride and pH of reaction medium was found 6.0.

3.1.2. Effect of gold nanoparticles solution volume
Maximum absorbance values were obtained using 3ml of gold nanoparicles solution.

3.1.3. Effect of temperature and time of heating

Standing for 15, 40 and 50 min. at room temperature was sufficient to produce maximum color intensities for pipazethate hydrochloride, fenoterol hydrobromide and clopidogrel hydrogen sulphate, respectively.

3.2. Method validation

3.2.1. Linearity

Under the described experimental conditions standard calibration curves with good linearity for pipazethate hydrochloride, fenoterol hydrobromide and clopidogrel hydrogen sulphate were constructed by plotting absorbance against concentration.

A linear correlation was found. The range of concentration, molar absorbtivity, correlation coeffiecient, intercept and slope of the calibration curve were calculated. Also relative standard deviation, analytical standard error, detection and quantification limits were calculated and listed in (Tables 2, 3).

Table 2 Spectral data for determination of pipazethate hydrochloride, fenoterol hydrobromide and clopidogrel hydrogen sulphate using citrate-capped gold nanoparticles (Au NPs)

Parameter	*Pipazethate hydrochloride*	*Fenoterol hydrobromide*	*Clopidogrel hydrogen sulphate*
Linearity range ($\mu g\ ml^{-1}$**)**	0.35 – 0.65	0.2 – 1.6	0.8 – 8.0
Apparent molar absorptivity*($mol^{-1}cm^{-1}$**)**	3.61×10^5	2.64×10^5	5.80×10^4
Sandell's sensitivity (mg/ml per 0001A)	9.04×10^{-2}	6.87×10^{-2}	1.38×10^{-2}
Limit of detection LOD ($\mu g\ ml^{-1}$**)**	0.100	0.061	0.259
Limit of quantification LOQ ($\mu g\ ml^{-1}$**)**	0.303	0.186	0.784
Regression equation:**			
Slope (b)	1.295	0.3921	0.1012
Intercept (a)	- 0.5006	0.2339	0.1020
Correlation coefficient (r)	0.9997	0.9999	0.9999

**Calculated on the basis of the molecular weight of the drug.*

*** A=a + bc*

Table 3 **Determination of pipazethate hydrochloride, fenoterol hydrobromide and clopidogrel hydrogen sulphate using citrate-capped gold nanoparticles (Au NPs)**

Statistics	**Pipazethate hydrochloride**		**Fenoterol hydrobromide**		**Clopidogrel hydrogen sulphate**	
	Taken $\mu g\ ml^{-1}$	**Recovery*** %	**Taken** $\mu g\ ml^{-1}$	**Recovery*** %	**Taken** $\mu g\ ml^{-1}$	**Recovery*** %
	0.35	100.13	0.2	99.59	0.8	100.05
	0.4	100.08	0.4	101.44	1.6	100.67
	0.45	99.81	0.8	99.18	2.4	98.81
	0.5	99.70	1.0	98.98	3.2	98.51
	0.55	99.80	1.2	100.76	4.0	101.53
	0.6	100.92	1.4	100.58	4.8	100.46
	0.65	99.55	1.6	99.63	6.6	99.90
					8.0	99.80
Mean±S.D.	100.00±0.454		100.02±0.913		99.97±0.977	
N	7		7		8	
V	0.206		0.833		0.955	
S.D.	0.454		0.913		0.977	
R.S.D.	0.454		0.912		0.978	
S.E.	0.172		0.345		0.346	

3.2.2. Application

The validity of proposed methods was assessed by its application to the determination of the cited drugs in their pharmaceutical preparations. (Table4).

Student's t-test and F-test (at 95% confidence level) were applied to the results obtained compared with that obtained from reference method [3] for pipazethate hydrochloride and official methods [8] for fenoterol hydrobromide and clopidogrel hydrogen sulphate. Results showed that there are no significant differences between the proposed and reference or official methods. Results of different statistical treatment of the data are shown in (Table 5).

Table 4 Determination of pipazethate hydrochloride, fenoterol hydrobromide and clopidogrel hydrogen sulphate using citrate-capped gold nanoparticles (Au NPs) in their pharmaceutical formulations

Selgon tablets (Pipazethate hydrochloride)		Selgon drops (Pipazethate hydrochloride)		Bronotrol Syrup (Fenoterol HBr)			Sigmagrel tablets (Clopidogrel hydrogensulphate)		
Taken µg ml^{-1}	**Recovery* %**	**Taken** µg ml^{-1}	**Recovery* %**	**Taken**	**Added**	**Recovery* %**	**Taken**	**Added**	**Recovery* %**
				µg ml^{-1}			µg ml^{-1}		
0.35	101.91	0.35	99.53	0.2	-	98.32	0.8	-	100.04
0.4	101.51	0.4	99.56		0.2	99.59		0.8	101.28
0.45	101.77	0.45	98.19		0.4	99.53		2.4	98.81
0.5	100.01	0.5	99.08		0.6	100.78		3.2	101.28
0.55	101.69	0.55	101.12		0.8	99.18		4.0	100.05
0.6	101.35	0.6	99.71		1.0	101.79		4.8	99.43
0.65	99.71							5.6	99.52
Mean ±S.D.	101.13 ±0.894	99.53 ±0.954		100.17 ± 1.086			100.06 ± 1.023		
N	7	6		5			6		
V	0.798	0.910		1.179			1.047		
S.D.	0.894	0.954		1.086			1.023		
S.E.	0.338	0.390		0.486			0.418		

** Mean of three different experiments*

Table 5 Statistical data for determination of pipazethate hydrochloride, fenoterol hydrobromide and clopidogrel hydrogen sulphate using citrate-capped gold nanoparticles(Au NPs)

Item	Pipazethate hydrochloride		Fenoterol HBr		Clopidogrel hydrogensulphate	
	Reference Method	**Reported method**	**Official Method**	**Reported method**	**Official method**	**Reported method**
Mean ±S.D.	100.05±0.604	100.00±0.454	99.08±1.209	100.02±0.913	99.10±1.091	99.97±0.977
N	8	7	4	7	3	8
V	0.360	0.206	1.460	0.833	1.190	0.955
S.D.	0.604	0.454	1.209	0.913	1.091	0.977
t		0.064 (2.160)*		0.640 (2.262)*		0.727 (2.262)*
F		1.747 (3.870)*		1.753 (4.760)*		1.246 (4.740)*

**Theoretical values of t and F at p = 0.05*

3.2.3. Accuracy and precision

Accuracy and precision were carried out by six determinations at two different concentrations of the four drugs in the same day (intra-day), and in six different days (inter-day). Percentage relative standard deviation (R.S.D. %) as precision, and percentage relative error (Er %) as accuracy of the suggested method were calculated. The percentage relative error calculated using the following equation:

Er % = [(founded − added) / added] × 100

The results of accuracy and precision (Table 6), show that the proposed methods have good repeatability and reproducibility.

Table 6 The intra-day and inter-day accuracy and precision data for determination of pipazethate hydrochloride, fenoterol hydrobromide and clopidogrel hydrogen sulphate using citrate-capped gold nanoparticles (Au NPs)

	Intra-day					Inter-day				
	Taken, µg ml^{-1}	**Found,** µg ml^{-1}	**Recovery *,** %	**RSD,** %	**Er,** %	**Taken,** µg ml^{-1}	**Found,** µg ml^{-1}	**Recovery *** %	**RSD,** %	**Er** %
Pipazethate hydrochloride	0.5	0.499	99.82	0.988	-0.18	0.5	0.499	99.80	1.216	-0.20
	0.6	0.604	100.72	0.840	0.72	0.6	0.605	100.79	0.973	0.79
Fenoterol hydrobromide	0.8	0.796	99.50	1.543	-0.50	0.8	0.796	99.44	1.885	-0.56
	1.6	1.593	99.53	0.915	-0.47	1.6	1.593	99.53	1.122	-0.47
Clopidogrel hydrogensulphate	4	4.045	101.12	1.213	1.12	4	4.063	101.57	1.309	1.57
	8	7.956	99.45	0.992	-0.55	8	7.959	99.49	1.014	-0.51

** Mean of six different experiments.*

CONCLUSION

In this work spectrophotometric method was successfully applied to determine pipazethate hydrochloride, fenoterol hydrobromide and clopidogrel hydrogen sulphate using citrate-capped nanoparticles (Au NPs). The proposed method is simple, sensitive, and inexpensive for their determination. This analytical protocol may be important green method for monitoring and optical detection of the studied drugs in pure and pharmaceutical dosage forms.

Acknowledgements
The work was financially supported by Faculty of pharmacy, Zagazig University, Egypt. Special thanks to analytical chemistry department. The authors wish to express their thanks to Regional Center for Mycology and Biotechnology (RCMB) at Al-Azhar University.

REFERENCES

[1] SC Sweetman. Martindale-The Complete Drug Reference, Thirty-fifth Edition, The Pharmaceutical Press, London, **2007**
[2]R El-Shiekh; F Zahran; AA Gouda. *Spectrochim. Acta Part A.*, **2007**, 66, 1279-1287.
[3] MM Hosny. *Int. J. Inst. Sci.*, **2013**, 2(2), 8-14.
[4] NT Abdel-Ghani; AF Shoukry; RM El Nashar. *Analyst*, **2001,** 126, 79-85.
[5] YS El-Saharty; NA El-Ragehy; HM Abdel-Monem; MI Abdel-Kawy. *J. adv. Res.*, **2010**, 1, 71-78.
[6] GM Hadad; R Abdel Salam; AS Emara. *J. Liq. Chromatogr. Relat. Technol.*, **2011**, 34, 1850-1869.
[7] O Ismaiel; M Hosny; G Ragab. *Asian Journal of Pharmaceutical and Clinical Research*, **2012**, 5(3), 215-2019.
[8]The British Pharmacopoeia**,** Volumes II and III, Her Majesty's Stationery Office, London, UK. **2013**
[9] S Shen; J Ouyang; WRG Baeyens, JR Delanghe, Y Yang. *J Pharm Biomed Anal.*, **2005**, 38(1), 166-172.
[10] M Sanghvi; A Ramamoorthy; J Strait; IW Wainer; R Moaddel. *J Chromatogr B.*, **2013**, 933, 37-43.
[11] T Ullrich; D Wesenberg; C Bleuel; G Krauss; MG Schmid; M Weiss; G Guebitz. *Biomed Chromatogr.*, **2010,** 24(10), 1125-1129.
[12] A Shalaby; H Salem; AA Kheir. *Scientia Pharmaceutica.*, **1997**, 65(3), 143-154.
[13] F Belal; HA AL-Malaq; A.A AL-Majed. *J Pharm Biomed Anal.*, **2000**, 23(6), 1005-1015.
[14] Y El-Shabrawy; F Belal; M Sharaf El-Din; S Shalan. *Il Fármaco***., 2003,** 58(10), 1033-1038.
[15] M Eid. *J. Chin. Chem. Soc***., 2007**, 54(3), 613-617.
[16] S Suhas Gurav; R Rohan Tembare; V Vishali Salunkhe; GP Devprakash. *American Journal of Pharm Tech Research*, **2011**, 1(4), 258-263.
[17] L Kalyan; HK Kunturkari. *Int. J. Pharm. Pharm. Sci***., 2013**, 5(3), 593-598.
[18] J Sippel; LL Sfair; EE Schapoval; M Steppe. *J AOAC Int.*,**2008**, 91(1), 67-72.
[19] N Sultana; MS Arayne; KA Ali1; M Nawaz. *J. Chromatogr. Sci.*, **2011**, 49, 165-169.
[20] R Sathiyasundar; K Valliappan. *Int. J. Pharm. Pharm. Sci.*, **2014**, 6(11), 279-283.
[21] D Antić; S Filipić; D Agbaba. *Chromatographica acta.*, **2007**, 18, 199-206.
[22] MA El-Sayed. *Acc. Chem. Res.*, **2001**, 34, 257-264.
[23] SJ Chen; YF Huang; CC Huang; KH Lee; ZH Lin; HT Chang. *Biosens. Bioelectron.*, **2008**, 23,1749-1753.
[24] M Amjadi; A Samadi. *J. Food Drug Anal.*, **2011**, 19(4), 410-417.
[25] Y Youmie Park; AR Im; YN Hong; CK Kim; YS Kim. *J. Nanosci. Nanotechnol.*, **2011**,11(9), 7570-2578.
[26] VV Apyari; SG Dmitrienko; VV Arkhipova; AG Atnagulov; YA Zolotov. *Anal. Methods.*, **2012,** 4(10), 3193-3199.
[27] MR Hormozi-Nezhad; E Seyedhosseini; H Robatjazi. *Scientia Iranica F***., 2012,** 19(3), 958-963.
[28] ARM Salcedo; FB Sevilla. *Philippine Science Letters*, **2013**, 3(1), 90-96.