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**Research Article**

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# Selective spectrophotometric methods for the determination of azithromycin in pharmaceutical formulation

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**ABSTRACT**

*The present study describes four simple, rapid, accurate, selective and sensitive spectrophotometric methods for the assay of azithromycin (AZM), a macrolide antibiotic, in bulk drug and pharmaceutical formulation. The methods are based on the formation of yellow ion-pair complex between the AZM and four sulphonphthalein dyes, namely, bromocresol green (BCG), bromocresol purple (BCB), bromophenol blue (BPB), bromothymol blue (BTB) in acetate-acetic acid buffer solution of PH 3.0 or 3.5. The ion-pair complex formed was extracted with chloroform and the absorbance was measured at 418, 409, 415 and 414 nm for BCG, BCB, BPB and BTB methods, respectively. Under the optimized conditions, beer's law is obeyed over the concentration ranges of 2-20, 2-18, 2-12 and 2-14 $\mu gmL^{-1}$ for BCG, BCB, BPB and BTB methods, respectively. The molar absorptivity, sandell sensitivity, detection and quantification limits are also calculated. The methods were validated for intra-day and inter-day accuracy and precision, selectivity and robustness and ruggedness. The proposed methods were applied successfully to the determination of AZM in their pharmaceutical formulations and the results were in good agreement with those obtained by the official method. The accuracy and reliability of the proposed methods were further ascertained by recovery studies via standard addition technique.*

**Keywords:** azithromycin; sulphonphthalein dyes; ion-pair complex; spectrophotometry; pharmaceutical analysis.

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## INTRODUCTION

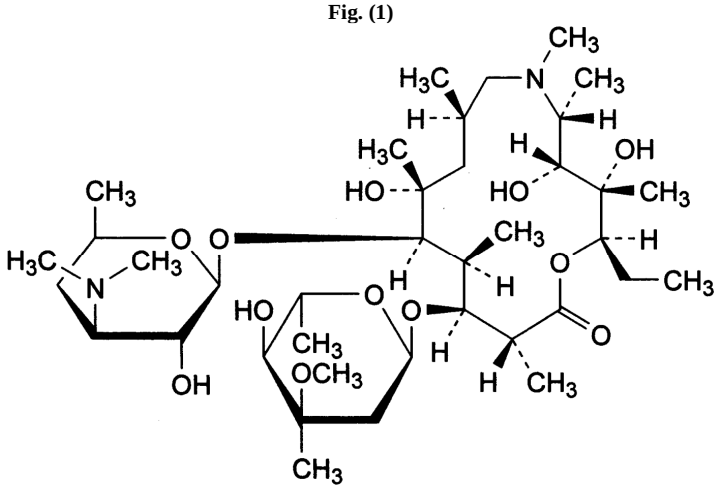
Azithromycin (AZM), fig. (1), chemically known as 9-deoxo-9a-aza-9a-methyl-9a-homoerythromycinis an antibiotic discovered by a Croatian group of researches, initially named XZ-450. It was developed by PLIVA, in the USA, and had its approval for clinical use in 1991 [1]. Azithromycin is active against some gram-positive and gram-negative micro-organisms and functions by binding to the 50S subunit of the bacterial ribosome. This action influences the microbial protein synthesis by preventing transpeptidation and translocation processes. Thus azithromycin has been to treat respiratory infection, skin and soft tissues infections and some sexually transmitted disease [2]. The drug is official in British pharmacopeia [3] which describes a liquid chromatographic method using 60 volumes of acetonitrile and 40 volumes of a 6.7 $gmL^{-1}$ solution of di-potassium hydrogen phosphate adjusted to pH 11 with a 560 $gmL^{-1}$ solution of potassium hydroxide as a mobile phase and an octadecylsilyl vinyl polymer as a stationary phase.

The literature survey reveals that several technique are available for the determination of AZM in pharmaceutical and include high-performance liquid chromatography [4-14], thin-layer chromatography [15], micellar liquid chromatography[16], voltammetry [17-20] Fourier transform infrared (FTIR) [21, 22] flow-injection chemiluminescence [23], flow-injection analysis and amperometric detecting [24,25], fluorescence [1],

spectrofluorometry [26-28], UV-spectrophotometry [29-31], derivative spectroscopy [32], visible spectrophotometry [33-48], conductometry [48], potentiometry [49,50] and flow injection spectrophotometry [51].

The most widely used technique for the assay of AZM in pharmaceutical has been visible spectrophotometry and methods based on such divers color reactions as oxidation of AZM with potassium permanganate [33, 40], ion-pair complexation [34,36], formation of binary complex [35], charge transfer reaction [37,39,41-43,45], formation of a red colored chromogen with ferric chloride and 1,10-phenanthroline [38], the acidic hydrolysis of AZM [44], reaction of AZM with 2,4-dinitrophenylhydrazine [46], reaction of AZM with sodium 1,2-naphthoquinone-4-sulphonate (NQS) [47] and formation of ternary complex [48], however, many of the above methods suffer from one or the other disadvantage like poor sensitivity [36,37 ,39,42 ,46], poor selectivity [33, 40], heating step [43,44] and use of expensive chemicals [41,43,45].

**Fig. (1)**



Extractive spectrophotometric procedure has received considerable attention for the quantitative determination of many organic compounds of pharmaceutical interest [52, 53]. This technique depends on the reaction of the drug that has basic cationic nitrogen and an anionic dye at a suitable pH, where a highly colored ion-pair complex is formed. However, no reports have appeared dealing with the extractive spectrophotometric method for the determination of AZM in drug forms so, far using sulphonphthalein acid dyes. Therefore, the aim of the present study was directed to develop four accurate, selective, precise and inexpensive procedure for the determination of AZM in pharmaceuticals based on ion-pair complex formation using four sulphonphthalein dyes, namely bromocrezol green (BCG), bromocresol purple (BCB), bromophenol blue (BPB) and bromothymol blue (BTB) as reagent.

## EXPERMENTAL SECTION

### Instruments and material

An optimum UV-VIS spectrometer (SP-3000 plus) (Tokyo, Japan) equipped with 1 cm matched quartz cells was used for all absorption spectral measurement. A Hanna pH-meter instrument (pH 211) (Romania) was used for checking the pH of Buffer solutions.

Pharmaceutical grade azithromycin (AZM) was provided by biopharmaceutical industries company, Sana a, Yemen. The following pharmaceutical preparations was purchased from commercial source in the local market and subjected to analysis. Azithromycin tablets 500mg from Alpha, Aleppo pharmaceutical industries company, Cairo, Egypt and Zisrocin capsules 500mg from Egypt pharma.

6 th. of October, Egypt.

### Reagent and chemicals

All reagents used were of analytical reagent grade, all solvents were of spectroscopic grade and bi-distilled water was used throughout the study.

A stock standard solution of 100 µg $mL^{-1}$ AZM was prepared by dissolving the appropriate weight of pure drug in least amount of ethanol and made up to 100 ml with bi-distilled water. Bromocrezol green (BCG), bromocresol purple (BCB), bromophenol blue (BPB) and bromothymol blue (BTB) (BDH Chemicals LTD, Poole, England) were used without further purification. Stock solution of 0.1 % (w/v) BCP, BCP, BPB and BTB were prepared by

dissolving the appropriate weight of dyes in 10 ml ethanol and diluted to 100 ml with bi-distilled water. Series of buffer solutions of KCl-HCl (pH 1.5-4.2), NaOAc-HCl (pH 1.99-4.92), NaOAc-AcOH (pH 2.5-5.6) and potassium hydrogen phthalate-HCl (pH 2.0-7.0) were prepared following the standard methods [54], freshly prepared solution were always used.

**Recommended procedure**

The stoichiometry of the studied drugs and the reagents under consideration in the complex was determined by the molar ratio method [55]. Into a series of separating funnels (0.2-2, 0.2-1.8. 0.2-1.2 and 0.2-1.4) ml aliquots of standard 100 µg $mL^{-1}$AZM solutions were transferred using a micro burette for BCG, BCP, BPB and BTB, respectively. The total volume in each separating funnel was adjusted to 2 ml by adding distilled water. To each funnel was added 2 ml acetate buffer of pH 3.0 for BPB and 2ml acetate buffer of pH 3.5 for BTB. Then 2.0 ml of 0.1 % (w/v) dye was added to each funnel. The content was mixed well and the formed ion-pair complex was extracted twice with 10 ml of chloroform after shaking for 2.0 min. the two phases were allowed to separate and the chloroform layer was dried over anhydrous sodium sulphate. The absorbance of yellow colored ion-pair complex was measured at 418, 409, 415 and 414 nm for BCG, BCP, BPB and BTB, respectively, against the corresponding reagent blank.

**Procedure for commercial tablets**

The contents of ten tablets or capsules each containing 500 mg of AZM were weighted and finely powdered. An amount of powder equivalent to 10 mg of AZM was accurately weighted and transferred to 100 ml volumetric flask and dissolved in 10 ml ethanol followed by the addition of 50 ml of distilled water. The solution was shaken thoroughly for about 10 min. diluted to mark with distilled water, mixed well and filtered using a whatman No. 42 filter paper. First 10 ml portion of the filtrate was rejected and a suitable aliquot of the filtrate (containing 100 µg $mL^{-1}$ AZM) was used for assay by the recommended procedure described above.

## RESULTS AND DISCUSSION

**Absorption spectra**

The nitrogenous drugs are present in positively charged protonated forms and anionic dyes of sulphonpthalein group present mainly in anionic form at pH ≥ 3. So when treated with an acid dye at pH of acidic buffers solution, a yellow ion-pair complex which is extracted with chloroform is formed. The absorption spectra of the ion-pair complexes, which were formed between AZM and each of BCG, BCP, BPB and BTB were measured in the range 350–550 nm against the blank solution and shown in (Fig. 2).

The ion-pair complexes show maximum absorbance at 420, 410, 414 and 417 nm for AZM using BCG, BCP, BPB and BTB, respectively. The optimum reaction conditions for determination of the ion-pair complexes were established. Then linearity, accuracy, precision, sensitivity, and stability of proposed methods were described **[56, 57]** and these developed methods applied to pharmaceutical preparations and obtained results evaluated statistically.

**Method validation**

**Analytical parameters**

Under optimum experiment conditions for AZM determination, a linear relation was found between the absorbance and concentration of AZM in the ranges of 2-20, 2-18, 2-12 and 2-14 µg $mL^{-1}$ for BCG, BCB, BPB and BTB methods respectively. Beers law is obeyed and the equation of the lines being:

| | |
|---|---|
| Y= 0.0048 + 0.0425 X | for BCG method |
| Y= 0.0088 + 0.0338 X | for BCG method |
| Y= o.oo88 + 0.0541 X | for BPB method |
| Y= o.oo37 + 0.0345 X | for BTB method |

Where Y is the absorbance and X is concentration in µg $mL^{-1}$. The linearity of calibration graphs was proved by high values of the correlation coefficient ® and the small values of the y-intercept of the regression equations. The molar absorptivity, sandall's sensitivity, limits of detection and quantification of the proposed methods were also calculated and recorded in table **1.**

**Accuracy and precision**

In order to determine of the proposed method, solution containing four different concentration of AZM (within the working limits) were prepared and analyzed in six replication and the analytical results were summarized in table**1**. The low values of the percentage relative standard deviation ( % R.S.D ≤ **0.70**) and the percentage relative errors (% R.E ≤ **0.078**) indicate the high precision and the good accuracy of the proposed method. RSD (%) and RE (%)

values were obtained within the same day to evaluate repeatability (intra-day precision) and over five days to evaluate intermediate precision (inter-day precision)

**Fig. 2. Absorption spectrum of ion-associate complexes of (10 µg mL$^{-1}$) AZM with 0.1 % (w/v) of BCG, BCP, BPB and BTB reagents**

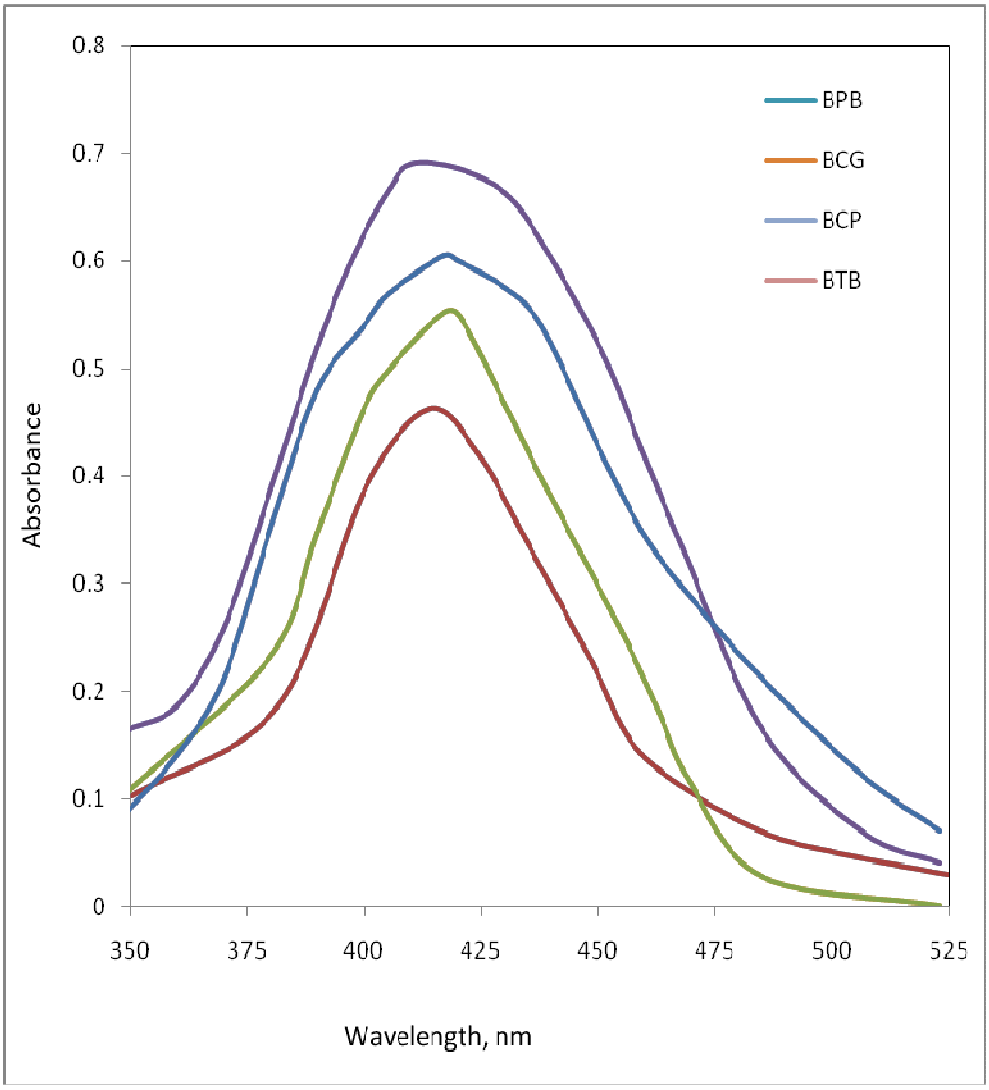



**Table 1. Statistical analysis of calibration graphs and analytical data in the determination of AZM using the proposed methods (*n* = 6)**

| Parameters | Proposed methods | | | |
|---|---|---|---|---|
| | **BCG** | **BTB** | **BCP** | **BPB** |
| **Wavelengths, $\lambda_{max}$ (nm)** | 418 | 414 | 409 | 415 |
| **pH** | 3.0 | 3.0 | 3.0 | 3.0 |
| **Beer's law limits (µg mL$^{-1}$)** | 2.0-20 | 2.0-14 | 2.0-18 | 2.0-12 |
| **Molar absorptivity ε, (L/mol$^{-1}$ cm$^{-1}$) x 10$^4$** | 1.320 | 1.087 | 1.131 | 1.683 |
| **sandall's sensitivity, ( µg cm$^{-2}$)** | 24.35 | 29.55 | 28.42 | 19.09 |
| **log $K_f$** | 5.13±0.14 | 5.22±0.06 | 4.68±0.21 | 5.08±0.07 |
| Regression equation ($y$) [a] | | | | |
| **Intercept ($a$)** | -0.0048 | -0.0037 | 0.0088 | -0.0086 |
| **Slope (b)** | 0.0425 | 0.0345 | 0.0338 | 0.0541 |
| **Correlation coefficient ($r$)** | 0.9999 | 0.9998 | 0.9997 | 0.9999 |
| **LOD (µg mL$^{-1}$)** | 0.15 | 0.16 | 0.23 | 0.14 |
| **LOQ (µg mL$^{-1}$)** | 0.50 | 0.53 | 0.77 | 0.47 |
| **RSD%** | 1.084 | 0.70 | 1.14 | 0.8698 |
| **RE%** | 0.736 | 1.196 | 0.9128 | 0.6875 |
| **t-test** [b] | 0.283 | 0.394 | 0.166 | 0.078 |
| **F- test** [b] | 1.44 | 1.85 | 1.07 | 1.64 |

*LOD, limit of detection; LOQ, limit of quantification; ε ,molar absorptivity.*
*[a] $y = a + bC$, where C is the concentration in µg mL$^{-1}$, y is the absorbance units.*
*[b] The theoretical values of t and F at P= 0.05 are 2.31 and 6.39, respectively.*

**Selectivity**

In order to evaluate the selectivity of the proposed methods for the analysis of AZM in pharmaceutical formulation, a placebo blank and synthetic mixture analysis was done. From the placebo blank analysis, it was confirmed that change in the absorbance with respect to the reagent blank was caused only by the analyte. The effect of the presence of the excipients, such as talc, starch, glucose, sodium alginate, calcium gluconate, sodium chloride, titanium dioxide and magnesium stearate was tested for possible interference in the assay synthetic mixture analysis and no interference was observed from these excipients.

**Application to analysis of pharmaceutical formulation**

The proposed methods were successfully applied to the determination of AZM in formulation (Azithromycin tablets 500 mg, xithrone tablets 500mg tablets and zisrocin capsules 500mg). The obtained results are showed in table 2 and were compared with those obtained by the reference method [27] by applying the students T-test and F-test at 95% confidence level. As can be seen from table 3, the calculated t and f values at 95% confidence level did not exceed the tabulated values of 2.78 and 6.39, respectively, indicating that there were no significance difference between the proposed methods and the reference methods with respect to accuracy and precision.

The accuracy and validity of the proposed methods were further confirmed by performing recovery experiment through the standard addition procedure. Pre-analyzed tablet or capsule powder was spiked with known amounts of pure AZM at three different concentration levels (50, 150, 200% of the quantity present in the tablet or capsule powder) and the total was measured by the proposed methods. The results of this study are presented in table 000 indicate that the excipiente present in the formulation did not interfere in the assay.

**Table 2. Determination of the studied drugs in their pharmaceutical dosage forms applying the standard addition technique**

| Sample | Taken | Added | Proposed methods | | Recovery [a] % | |
|---|---|---|---|---|---|---|
| | (µg mL$^{-1}$) | (µg mL$^{-1}$) | BCG | BCP | BPB | BTB |
| Azithrocine | 4 | 2 | 99.92 | 100.01 | 99.89 | 99.95 |
| (500 mg AZM/ tablet) | | 6 | 99.85 | 99.98 | 99.56 | 100.06 |
| | | 8 | 100.03 | 100.08 | 99.9 | 99.91 |
| Xithrone | 4 | 2 | 100.05 | 100.02 | 99.87 | 99.93 |
| (500 mg AZM/ tablet) | | 6 | 99.94 | 99.85 | 99.9 | 100.03 |
| | | 8 | 99.9 | 99.96 | 99.95 | 99.92 |
| Zisrocin | 4 | 2 | 99.94 | 99.97 | 99.65 | 99.86 |
| (500 mg AZM/ capsule) | | 6 | 100.05 | 100.03 | 99.73 | 99.9 |
| | | 8 | 100.09 | 99.88 | 99.95 | 99.93 |

[a] *Average of six determination*

**Table 3. Determination of the studied drugs in pharmaceutical dosage forms**

| Samples | Official methods (n = 5) | Proposed methods (n=6) Recovery [a] ± SD % | | | |
|---|---|---|---|---|---|
| | | BCG | BCP | BPB | BTB |
| Azithrocine | 99.70 ± 0.16 | 99.86 ± 0.19 | 99.55 ± 0.18 | 99.80 ± 0.22 | 99.91 ± 0.20 |
| (500 mg AZM/ tablet) | 0.00512 | *t** = 1.51 | *t*= 1.46 | *t*= 0.87 | *t*= 1.93 |
| | | *F**= 1.41 | *F*= 1.27 | *F*= 1.89 | *F*= 1.56 |
| Xithrone | 99.89 ± 0.61 | 100.02 ± 0.72 | 99.94 ± 0.68 | 99.63 ± 0.81 | 100.06 ± 0.52 |
| (500 mg AZM/ tablet) | | *t*= 0.324 | *t*= 0.13 | *t*= 0.606 | *t*= 0.492 |
| | | *F*= 1.39 | *F*= 1.24 | *F*= 1.76 | *F*= 1.38 |
| Zisrocin | 99.95 ± 0.37 | 100.09 ± 0.43 | 99.90 ± 0.50 | 100.11 ± 0.29 | 99.82 ± 0.48 |
| (500 mg AZM/ capsule) | 0.02738 | *t*= 0.583 | *t*= 0.19 | *t*= 0.79 | *t*= 0.507 |
| | | *F*= 1.35 | *F*= 1.83 | *F*= 1.63 | *F*= 1.68 |

[a] *Average of six determinations.*

** The theoretical values of t and F at P= 0.05 are 2.31 and 6.39, respectively.*

## CONCLUSION

Unlike GC and HPLC procedures, the spectrophotometer is simple and is not of high cost. The importance lies in the chemical reactions upon which the procedures are based rather than upon the sophistication of the instrument. This aspect of spectrophotometric analysis is of major interest in analytical pharmacy since it offers distinct possibility in the assay of a particular component in complex dosage formulations. The reagents utilized in the proposed methods are cheaper, readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation. The method is unaffected by slight variations in experimental conditions such as pH and reagent concentration. Moreover, the methods are accurate, reproducible, adequately sensitive and free from interference by common additives and excipients. The wide applicability of the new procedures for routine quality control is well established by the assay of AZM in pure form and in pharmaceutical preparations.

**REFERENCES**

[1] D. L. Massart, B. G. M. Vandeginste, S. N. Deming, Y. Michotte and F. Kaufman, "*Chemometrics: a textbook*" Chap. 18, Elsevier, Amsterdam, **1988**.pp. 293 – 304,
[2] Elks, J. and Ganellin, C. R, Dictionary of Drugs, Chapman and Hall, London, **1991**.
[3] Martindale, The complete drug reference. Sean, C. S., Pharmaceutical Press, London-Chicago, (**2009**), 36th edn, 207.
[4] The British Pharmacopoeia (BP), Her Majesty's Stationary Office London, (**2009**), Vol. I and II, 493.
[5] Harry G. Britton "Analytical Profile of Drug Substances and Excipients ", Academic Press, **1996**, 24, 45-84.
[6] *Martindale*, The complete drug reference. Sean, C. S., Pharmaceutical Press, London-Chicago, (**2009**), 36th edn, 248.
[7] *The British Pharmacopoeia (BP)*, Her Majesty's Stationary Office London, (**2009**), Vol. I and II, 1406.
[8] Martindale, The complete drug reference. Sean, C. S., Pharmaceutical Press, London-Chicago, (**2009**), 36th edn, 331.
[9] The British Pharmacopoeia (BP), Her Majesty's Stationary Office London, (**2009**), Vol. I and II,. 5252
[10] Rufino, J. L., Pezza, H. R., Pezza, L., *Anal. Sci*., (**2008**), 24, 871.
[11] Jing, M. A., Tang, Q., Zhou, X. M., *Chin. J., Drugs*, (**2008**), 17, 22.
[12] Huakan, L., Yanqing, Z., Yuhua, W., Janfeng, K., *Chin. J. Anal. Chem*., (**2004**), 32, 598.
[13] Huang, W., Liu, X. J., Zhao, F. L., Guang Pu Xue Yu Guang Pu Fen Xi, (**2006**), 26, 913.
[14] Kelani, K., Bebawy, L. I., Abdel-Fattah, L., Ahmed, A. K. S., *Anal. Lett*., (**1997**), 30, 1843.
[15] Mohamed, I. W., Mohamed, S. R., Manal, I. E., Mona, E. F., *J. AOAC. Int*., (**2007**), 90, 1579.
[16] *Abdelmageed, O. H., J. AOAC. Int*., (**2007**), 90, 364.
[17] Rachidi, M., Elharti, J., Digua, K., Cherrah, Y., Bouklouze, A., *Anal. Lett*., (**2006**), 39, 1917.
[18] Sultana, N., Arayna, M. S., Hussain, F., Fatima, A., *Pak. J. Pharm. Sci*., (**2006**), 19, 94.
[19] Suhagia, B., Shah, S., Rathod, I., Patel, H., Doshi, K., *Ind. J. Pharma. Sci*., (**2006**), 68, 242.
[20] Sivasubramanian, L., Mervin, M. A., Jayashankar, L., Ramu, P., Raja, T. K., *Ind. J. Pharm. Sci*., (**2004**), 66, 249.
[21] Kuchekar, B. S., Shinde, D. B., *Ind. Drugs*, (**2001**), 38, 439.
[22] El-Rabbat, N., Askal, H. F., Khashaba, P. Y., Attia, N. N., J. *AOAC. Int*., (**2006**), 89, 1276.
[23] Khashaba, P. Y., *J. Pharm. Biomed. Anal*., (**2002**), 27, 923.
[24] Chen, C., Zhua, L. F., *Fenxi Shiyanshi*, (**2002**), 21, 37
[25] Song, Z., Wang, C., *Biomed. Chem*., (**2003**), 11, 5375.
[26] Yang, Z. Y., Wang, L., Tang, X., *J. Pharm. Biomed. Anal*., (**2009**), 49, 811.
[27] Shaikh, K. A., Patil, S. D., Devkhile, A. B., *J. Pharm. Biomed. Anal*., (**2008**), 48, 1481.
[28] Shi, Y., Yao, G. W., Ma, M., Fenxi-Huaxue, (**2005**), 33, 512.
[29] Sukanya, S., Julraht, K., *Southeast Asian, J. Trop, Med. Public Health*, (**2008**), 39, 978.
[30] Xu, F., Zhang, Z. J., Bian, Z. W., Tian, Y., Jiao, H. Y., Liu, Y., *J. Chromatogr. Sci.*, (**2008**), 46, 479.
[31] Liu, F., Xu, Y., Huang, J. C., Gao, S., Guo, Q. X., *Biomed. Chromatogr.*, (**2007**), 21, 1272.
[32] Chen, B.M., Liang, Y. Z., Chen, X., Liu, S. G., Deng, F. L., Zhou, P., *J. Pharm. Biomed. Anal*., (**2006**), 42, 480.
[33] Yuzuak, N., Ozden, T., Eren, S., Toptan, S., *Chromatographia*, (**2007**), 66, 115.
[34] Chen, L., Qin, F., Ma, Y. Y., Li, F. M., *J. Chromatogr B.*, (**2007**), 855, 255.
[35] Zhou, X. M., Liu, J. Gao, J. Yao, Q. S., Wang, W. Y., *Pharmazie*, (**2007**), 62, 255.
[36] Li, Z. J., Liu, K., Pei, Q., Li, B., Zhou, Y. L., Yaowu Fenxi Zazhi, (**2006**), 26, 726.
[37] Nirogi, R. V. S., Kandikere, V. N., Shukla, M., Mudigonda, K., Maurya, S., Boosi, R., Yerramilli, A., *Analytica Chimica Acta*, (**2005**), 553, 1.
[38] Barrett, B., Borek-Dohalsky, V., Fejt, P., Vaingatova, S., Huclova, J., Nemec, B., Jelinek, I., Anal. Bio. anal. Chem., (**2005**), 383, 210.
[39] Bahrami, G., Mirzaeei, S., Kiani, Amir., *J. Chromatogr., B.* (**2005**), 820, 277.
[40] Wilms, E., Trumpie, H., Veenendaal, W., Touw, D., *J. Chromatogr. B*, (**2005**), 814, 37.
[41] Kwiecien, A., Krzek, J., Biniek, L., *J. Planar Chromatogr. –Mod.* TLC., (**2008**), 21, 177.
[42] Palomeque, M. E., Ortiz, P. I., *Talanta*, (**2007**), 72, 101.
[43] Rachidi, M., Elharti, J., Digua, K., Cherrah, Y., Bouklouze, A., *Anal. Lett*. (**2007**), 40, 53.
[44] Araujo, J., Ortiz, R., Velasquez, W., *Port. Electrochim. Acta*, (**2006**), 24, 71.
[45] Avramov, I. M. L., Petrovic, S. D., Mijin, D. Z., Zivkovic, P. M., Kosovic, I. M., Drljevic, K. M., Jovanovic, M. B., *Electrochimica Acta*, (**2006**), 51, 2407.
[46] Kim, Y. H., Pothuluri, J. V., Cerniglia, C. E., *J. Pharm. Biomed. Anal*. (**2005**), 38, 390.
[47] Yunhua, W., Xiaobo J. S., *Bio. Elect. Chem*., (**2004**), 64, 91.
[48] Shah, J., Jan, M. R., Manzoor, S. J., *Chin. Chem. Soci*., (**2008**), 55, 1107.
[49] Rao,Y. S., Jitendrababu, V., Chowdary, K. P. R., Rao, J. V., *Ind. J. Pharm. Sci*., (**2003**), 65, 653.
[50] Sudheer, P., Ganapathi, P. A. V., *Ind. Drugs*, (**2001**), 38, 358.

[51] Hamdan, I. I., Mishal, A. M., *Saudi. Pharm. J*., (**2000**), 8, 191.
[52] Li, H., Xiao, J., *Fenxi Huaxue*, (**2005**), 33, 1327.
[53] Rao, Y.S., Murthy, T.K., Chowdary, K.P.R., Rao, J.V. L. N. S., *Ind. Drugs*, (**2002**), 39, 348.
[54] Rao, J. V. L. N. S., Rao, Y. S., Murthy, T. K., Sankar, D. G., *Asi. J. Chem.*, (**2002**), 14, 647.
[55] Srinivasa Rao, Y., Rajani Kumar, V., Seshagiri Rao, J. V. L. N., *Asi. J. Chem*., (**2002**), 14, 1791.
[56] Validation of Analytical Procedures, Methodology ICH Harmonised Tripartite Guideline, Having Reached Step 4 of the ICH Process at the ICH Steering Committee meeting on November 6, **1996**.
[57] Inczedy J., Analytical Applications of Complex Equilibria; Ellis Horwood Ltd: England, (**1976**) 137–143.
[58] Miller J. C., Miller J. N., Significance tests. In Statistics in Analytical Chemistry, 3rd Ed.; Ellis Horwood: Chichester, (**1993**) Chapter 3.