

Spectrophotometric Determination of Drugs and Pharmaceuticals by Cerium (IV) Amaranth Dye Couple

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Abstract: Simple, sensitive, accurate, and precise spectrophotometric methods for quantitative determination of drugs, viz., Darifenacin (DAR), Esmolol Hydrochloride (ESM), Montelukast Sodium (MON), Sildenafil citrate (SIL), Terbinafine (TER) and Tramadol Hydrochloride (TRA) were developed. The method of each drug depends upon oxidation of drugs by Ce (IV) (Excess) and estimating the amount of unreacted Ce (IV) by amaranth dye at 523nm. The calibration curves obeyed Beer's law over the concentration range of 1.4-7.0 $\mu\text{g ml}^{-1}$ (DAR), 2-14 $\mu\text{g ml}^{-1}$ (ESM), 2-10 $\mu\text{g ml}^{-1}$ (MON), 20-70 $\mu\text{g ml}^{-1}$ (SIL), 3-21 $\mu\text{g ml}^{-1}$ (TER) & 2-14 $\mu\text{g ml}^{-1}$ (TRA). The methods have been validated in terms of guidelines of ICH and applied to analysis of pharmaceuticals.

Keywords: Cerium (IV), Amaranth dye couple, drugs, Determination, UV-VIS Spectrophotometry

I. Introduction

1.1. Darifenacin

Darifenacin is chemically as (s)-2-{1-[2-(2, 3-dihydrobenzofuran-5-yl) ethyl 3-pyrrolidine]-2-diphenyl acetamide [Fig. 1(a)], is a M₃ receptor antagonist. It is used for treatment of overactive bladder with the symptoms of urinary frequency, urinary urgency, and urinary incontinence [1].

Because of its physiological significance several methods have been developed for its quantitative determination viz., PR-HPLC [2,3], HPTLC[4], Spectrophotometry [5,6] and Extractive spectrophotometry[7,8].

1.2. Esmolol hydrochloride

Esmolol hydrochloride (ESM), methyl 3-{4-[2-hydroxy-3-(isopropylamino) propoxy] phenyl}propionate hydrochloride, is an ultra-short acting adrenergic receptor antagonist used for the rapid control of heart rate in patients with atrial fibrillation or atrial flutter. Since ESM is widely used in the rapid control of heart rate, it is important to develop and validate analytical methods for its determination in pharmaceutical dosage form. Review of literature has revealed that few methods have been reported for the estimation of Esmolol hydrochloride. Most HPLC methods reported are useful in estimating Esmolol hydrochloride in human plasma [9-13] and biological fluids [14]. HPLC [15, 16] methods have been reported for determination of ESM in pharmaceutical injections.

1.3. Montelukast sodium

Montelukast sodium 2-[1-[(R)-[3-[2(E)-(7-chloroquinolin-2-yl) vinyl] phenyl] -3-[2-(1-hydroxy-1-methyl ethyl) phenyl] propyl – sulfanyl methyl] cyclo propyl] acetic acid sodium salt [Fig.1(c)]. It is an oral selective leukotriene receptor antagonist that inhibits the cysteinyl leukotriene cysLT1 and has been shown to be effective in the treatment of chronic asthma [17].

Several methods like Spectrophotometry for individual [18,19], combined dosage form [20], High-performance liquid chromatography [21,22], Capillary electrophoresis [23] and Voltametric determination [24] have been developed.

1.4. Sildenafil citrate

Sildenafil citrate (SIL) is designated chemically as 1- h[3- (6,7-dihydro-1-methyl-7-oxo-3-propyl-1-Hpyrazolo[4,3-d]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl]-4-methylpiperazine citrate and has the structural formula shown in [Fig. 1(d)]. It is used in oral therapy for erectile dysfunction, is a selective inhibitor of cyclic guanosine mono phosphate (cGMP) specific phosphodiesterase type 5 (PDE5) [25].

Some techniques have been developed for quantitative determination of SIL in pharmaceutical formulations. Some important ones are HPLC [26,27,28], flow-injection analysis with multiple pulse amperometric detection [29], atomic emission spectrometry [30], spectrophotometry [31,32] and LC-MS [33].

1.5. Terbinafine

Terbinafine [Fig.1(e)], chemically (E)-N-(6,6-dimethyl-2-hepten-4-ynyl)-N-Methyl-1-naphthalene

methanamine, is an allyl amine derivative with antifungal activity. The drug has been found to be a potent inhibitor of squalene epoxidase which is an enzyme present in fungal and mammalian cell systems important in ergo sterol biosynthesis [34].

Because TER is widely used, several methods have been employed to determine TER in pharmaceutical formulations and they include Potentiometry [35], HPLC [36], RP-HPLC [37] and Spectrophotometry [38,39].

1.6. Tramadol hydrochloride

Tramadol hydrochloride (TRA), chemically known as (1R, 2R) -rel-2- [(Dimethylamino) methyl]-1-(3-methoxyphenyl)cyclohexanol; (\pm)-cis-2 [Fig.1(f)], administered orally non-steroidal anti-inflammatory drug which possesses good analgesic properties and good tolerability profile in variety of painful conditions [40].

Some methods Spectrophotometry [41, 42], HPLC [43], UV-Spectrometry [44], Potentiometry [45,46] and Conductometry [47] have been developed for the determination Tramadol HCl.

Through survey of literature revealed that oxidative method of quantification of these drugs by Ce (IV) have been not reported yet, although the methods simple sensitive, precise and accurate [48, 49].

II. About The Method

Cerium (IV) is a good oxidizing agent like KMnO_4 , $\text{K}_2\text{Cr}_2\text{O}_7$ etc., it has been used for quantitative determination of drugs based on the oxidation of drugs. The spectrophotometric methods involved addition of excess Ce(IV) and un reacted cerium is estimated by suitable dyes, which should be oxidized by cerium viz., Indigo Carmine, Methyl Orange, Safranin-O and Xylene cyanol.

Amaranth dye is suitable for estimation of unreacted Ce (IV) absorbance at 523 nm.

III. Experimental

3.1. Apparatus

Spectral and absorbance measurements were made on a thermo electron corporation single beam U.V.-Vis spectrophotometer by using 1 cm quartz cells.

3.2. Materials and methods

All reagents used were of analytical-reagent grade and distilled water was used throughout the investigation.

3.2.1. Cerium (iv) solution

Cerium (IV) sulphate ($\text{CeSO}_4 \cdot 2\text{H}_2\text{O}$, 99.9 % pure) was prepared by dissolving 750 mg of chemical (merck, mumbai, india) in 2 N H_2SO_4 with the aid of heat and filtered using glass wool, and diluted to 250 ml with the same acid and standardized and cerium is standardized by ferrous ammonium sulphate and ferroin indicator. The solution was then diluted appropriately with 2 N H_2SO_4 to get working concentrations of 4.0×10^{-3} M (0.25%).

3.2.2. Amaranth dye

Aqueous solutions of 0.8×10^{-3} M of Amaranth dye was prepared by dissolving an appropriate weight of 0.0483 grams in 100 ml bi distilled water.

3.2.3. Sulphuric acid

Prepared by diluting the concentrated acid (Merck, Mumbai, India, Sp. gr. 1.84, 98.0 %) with water appropriately to get 2 N acid.

3.2.4. Preparation of drug solution

Standard drug solution ($200 \mu\text{gml}^{-1}$) was prepared by dissolving 20 mg of drug with distilled water to the mark in 100 ml standard flask. The stock solution was diluted appropriately to get the working concentration.

IV. Procedure

Aliquots of studied drugs containing 1.4-70.00 $\mu\text{g/ml}$ of drug were transferred into a series of 10 ml standard flasks using a micro burette. To each flask were added 1 ml of sulfuric acid (2N) and 1 ml of cerium (IV) ammonium sulfate solution ($250 \mu\text{g ml}^{-1}$). The content mixed and heated on water at $60 \pm 2^\circ\text{C}$ for 10 min, then the solution was cooled for room temperature. Finally, 1 ml of 0.02% of amaranth dye solution was added and diluted to the mark with doubly distilled water, mixed and absorbance of each solution measured at 523 nm

against water blank.

V. Assay Of Drug Pure Sample

To the test the accuracy and precision of the methods developed pure sample solutions containing drug in the Beer's Law limit were chosen. For this study 1.4-7.0 μgml^{-1} of DAR, 10-70 μgml^{-1} of ESM, 2-10 μgml^{-1} of MON, 20-70 μgml^{-1} of SIL, 3-21 μgml^{-1} of TER & 2-14 μgml^{-1} of TRA have been taken. To each of the solution 1 ml of 250 $\mu\text{g ml}^{-1}$ of cerium 1 ml of 2 N of H_2SO_4 were added the un reacted cerium is analyzed as described above using amaranth dye.

VI. Procedure For Analysis Of Pharmaceuticals

6.1. Darifenacin

Four tablets (VESIGARD, 15mg tablet⁻¹) were weighed and grounded. A quantity equivalent to 20 mg of darifenacin was transferred into a 100ml calibrated flask and the volume was finally diluted to the mark with distilled water, mixed well and filtered using a whatmann No.42 filter paper. First 10ml portion of filtrate was discarded and a suitable aliquot of the subsequent portion was diluted appropriately to get required concentration and the assay was completed according to the procedure described above.

6.2. Esmolol chloride

Three CLOL (Health Biotech : 50 mg 10 ml⁻¹) injections were taken and combined, diluted further to get required concentrations.

6.3. Montelukast sodium

Four tablet of MONTAIR (CIPLA : 10mg tablet⁻¹) were accurately weighed and finely powdered. The powder equivalent to 20 mg of MON was transferred into 100ml volumetric flask and dissolved in 0.2 M H_2SO_4 . then the solution was filtered using whatmann No:41 filter paper and further diluted with water to obtain working standard solution.

6.4. Sildenafil citrate

Four tablets of ALISIGA containing 25 mg each amounting about 100 mg of SIL was accurately weighed, dissolved in water and diluted to volume 100 ml calibrated flask. This solution was diluted stepwise to give a series of concentrations suitable for the construction of the calibration graph.

6.5. Terbinafine

To determine the content of Terbinafine in pharmaceutical preparations, four tablets of Tebina (lable claim : 25 mg/tablet) were weighed and finely powdered. A portion of the powder equivalent to 50mg. Terbinafine was stirred with 50 ml doubly distilled water and let stand for 10 minutes. The residue was filtered on Whatmann No.41 filter paper and wash with doubly distilled water. This solution was further diluted as necessary to complete the analysis concentration solutions for assay.

6.6. Tramadol hydrochloride

For the analysis of pharmaceutical formulations ten capsles (Dolotram: 20mg) were weighed, powered and equivalent to 10 mg of tramadol hydrochloride was transferred in to 100 ml volumetric flask. 60.0 ml of distilled water was added and ultrasonicated for 20 min, then made up to the mark with distilled water. The resulting solution was mixed and filtered through Whatmann filter paper no. 42. From the filtrate solution was diluted appropriately with distilled water in order to obtain working concentration of drug used for the analysis.

VII. Method Of Validation

The each method developed quantification of drugs has been validated in terms of precision, accuracy, limit of detection, limit of quantification, linearity, selectivity and ruggedness. Absorbance time curves were drawn, initial rate and fixed time methods were used to assess the recovery of the drug. To assess the precision each experiment was repeated at least 5 times and accuracy is estimated in terms of percent recovery and percent RSD. Excellent percent recovery and RSD being less than 2 for each drug demonstrates accuracy and precision of the methods. Further t-test and F-test values have also been calculated using a standard reference method. The closeness of t-test and F-test values is less than that they permissible range indicating high accuracy of the methods [Table 2].

As mentioned earlier limit of detection is the minimum limit that can be detected but not necessarily quantified is determined for each drug.

LOD is determined from the standard deviation of y-intercepts of regression lines of replicate

determinations.

$$\text{LOD} = 3.3 \text{ s/S}$$

Where s = standard deviation of intercept (n=6)

S = slope of linearity plot

LOQ the minimum concentration of analyst using calibration curve is also determined.

$$\text{LOQ} = 10\text{s/S.}$$

Limits of linearity of calibration curves are mentioned in the [Fig. 2] under the title Beer's law limit. To test the selectivity known excipients of each drug are added to the pure drug sample and recovery experiments were performed. Ruggedness is resistance of method for a small change in variables like instrument, and analyst or both to test the Ruggedness of the method absorbance data was collected using 3 different instrument and 2 analysts no significant changes were observed either by change of instrument or analyst hence the method may be taken as robust.

VIII. Factors Effecting Absorbance

8.1. Effect of acid concentration

To study the effect of acid concentration, different types of acids were examined (H_2SO_4 , H_3PO_4 and CH_3COOH) to achieve maximum yield of Redox reaction. The results indicated that the sulphuric acid was the preferable acid with Ce (IV) as oxidant. The reaction was performed in a series of 10 ml volumetric flask containing $8.0 \mu\text{gml}^{-1}$ of the cited drugs, different volumes (0.5–2.5 ml) of 2.0 N H_2SO_4 and 1 ml of Ce(IV) ($4.0 \times 10^{-3} \text{M}$) were added. After 5.0 min of heating time at $60 \pm 2^\circ\text{C}$ in a water bath, the solution was cooled for about 3.0 min, 1.0 ml of amaranth dye were added, then complete to 10 ml total volume with water. It was found that the maximum absorbance was obtained at 1 ml of 2 N H_2SO_4 . Above this volume, the absorbance decreased therefore, a volume of 1 ml of 2 N H_2SO_4 was used for all measurements.

8.2. Effect of heating time

In order to obtain the highest and most stable absorbance, the effect of heating time on the oxidation reaction of drugs were catalyzed by heating in a water bath at $60 \pm 2^\circ\text{C}$ for the periods ranging for 2.5-20 min. the time required to complete the reaction and maximum absorbance was obtained after 5.0 min of heating. After oxidation process, the solution must be cooled at least for 3.0 min before addition of dye.

8.3. Effect of oxidant concentration

When a study on the effect of Ce (IV) on color development was performed, it was observed that in both cases the absorbance increased with increase in the volume of Ce (IV). It reached maximum when 1 ml of $200 \mu\text{g ml}^{-1}$ Ce (IV) solution was added to a total volume of 10 ml for drugs solutions. The color intensity decreased above the upper limits. Therefore, 1 ml of $200 \mu\text{g ml}^{-1}$ Ce (IV) was used for all measurements.

8.4. Effect of dye concentration

In order to ascertain the linear relationship between the volume of added Ce (IV) and the decrease in absorbance of Amaranth dye, experiments were performed using 1 ml of 2 N H_2SO_4 with varying volumes of Ce (IV). The decrease in absorbance was found to be linear up to the 1 ml of $200 \mu\text{g ml}^{-1}$ Ce (IV) with optimum volume 1.0 ml of Amaranth dye for fixed concentration drug solution. The color was found to be stable up to 24 hours.

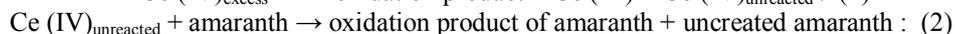
IX. Analysis Of Pharmaceuticals

To the test the applicability of the method developed solution of pharmaceutical tablets solutions containing drug in the Beer's Law limit were chosen. To assess the precision each tablet analysis was repeated at least 6 times and accuracy is estimated in terms of percent recovery and percent RSD. Excellent percent recovery and RSD being less than 2 for each drug demonstrates applicability of the methods for pharmaceutical analysis [Table 3]. Further t-test and F-test values have also been calculated using a standard reference method. The closeness of t-test and F-test values is less than that they permissible range indicating excellent applicability of the methods for pharmaceutical analysis [Table 4]. The excellent recovery studies indicate that methods developed can be applied to pharmaceutical analysis without hesitation.

X. Results And Discussion

The ability of cerium (IV) sulphate to oxidize drugs, and bleach the color of amaranth dye is the basis of the indirect spectrophotometric method developed here. In this method the drugs were reacted with a measured excess of cerium(IV) sulphate in acidic medium and the unreacted oxidant was determined by reacting with amaranth followed by absorbance measurement at 523 nm (scheme 1). The absorbance increased linearly with increasing concentration of drug, when increasing amounts of each drug were added to a fixed

amount of 0.25% of CAS, consumed the latter and there occurred a concomitant fall in its concentration. When fixed amount of the dye was added to decreasing amount of oxidant, an concomitant increase in the concentration of dye resulted. This was observed as a proportional increase in absorbance at the respective λ_{\max} with increasing concentration of each drug. One ml of 2N acid was used in the reaction, as this concentration was found ideal.



Measured spectrophotometrically at $\lambda_{\max} = 523 \text{ nm}$

Scheme 1: Reaction Scheme of the indirect determination of drug by oxidation with Ce (IV) sulphate

XI. Analytical Data

A linear correlation was found between absorbance at λ_{\max} and concentration ranges, and sensitivity parameters such as molar absorptivity, Sandal's sensitivity, detection limit and quantification limit are presented in Table 1. Regression analysis of Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a), correlation coefficient (r) and is also given in Table 1.

XII. Accuracy And Precision

The accuracy and precision of the methods were established by analyzing the pure drug solution at 6 different levels (with working limits). The relative error (%) which is a measure of accuracy & RSD (%) a measure of precision are summarized in Table 2 and reveal the high accuracy and precision of the methods.

XIII. Conclusion

The present study described the successful development of new, simple, sensitive, selective, accurate and rapid spectrophotometric method for the accurate determination of drugs each one in its pharmaceutical forms Cerium (IV) sulphate as the oxidizing reagent. There is no interference from additives and excipients. The method thus can be used in the determination of these drugs in pure and pharmaceutical formulations. So, it is the good alternative to the reported methods for the determination of these drugs.

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References

- [1] The Merck Index, 13th edition, Merck Research laboratories, White House station, NJ, 2001, Pg: 495.
- [2] Murthy, M. Vishnu; Krishnaiah, Ch.; Srinivas, K.; Rao, K. Srinivasa; Kumar, N. Ramesh; Mukkanti, K., Development and validation of RP-UPLC method for the determination of darifenacin hydrobromide, its related compounds and its degradation products using design of experiments, *Journal of Pharmaceutical and Biomedical Analysis* (2013), 72, 40-50.
- [3] Panda, Sagar Suman; Kumar, B. V. V. Ravi; Patel, Pinkal Kumar; Mohanta, Ganeswar, New stability indicating RP-UFLC method for determination of darifenacin hydrobromide in extended release tablet dosage form, *Journal of Pharmaceutical Education and Research* (2012), 3(2), 7-12.
- [4] Sathish, N. K.; Chethan, I. A.; Khandelwal, Pradeep V.; Shrestha, Ashwinee Kumar, Development and validation of rapid HPTLC method for determination of darifenacin hydrobromide in bulk drug and pharmaceutical dosage form, *Chemica Sinica* (2011), 2(3), 57-62.
- [5] Sathish, N. K.; Khandelwal, Pradeep V.; Chethan, I. A.; Shrestha, Ashwinee Kumar, UV spectrophotometric determination of darifenacin hydrobromide in bulk and pharmaceutical dosage forms, *Pharmacia Sinica* (2011), 2(2), 169-176
- [6] Sai Praveen, P.; Shaiba, M.; Swetha, A.; Aruna Jyothi, A.; Nishat, Amreen, Spectrophotometric methods for the determination of Darifenacin, *Research Journal of Pharmaceutical, Biological and Chemical Sciences* (2010), 1(4), 350-353.
- [7] Sai Praveen, P.; Jagathi, V.; Devala Rao, G.; Sudhakar Saibabu, G., Visible spectrophotometric methods for the determination of Darifenacin, *Research Journal of Pharmaceutical, Biological and Chemical Sciences* (2010), 1(2), 254-257.
- [8] Praveen, P. Sai; Jagathi, V.; Rao, G. Devala; Saibabu, G. Sudhakar, Extractive spectrophotometric methods for the determination of Darifenacin, *Oriental Journal of Chemistry* (2010), 26(1), 195-197.
- [9] Tang Y, He Y, Yao T and Zeng S. Simultaneous determination of the enantiomers of esmolol and its acid metabolite in human plasma by reversed phase liquid chromatography with solidphase extraction, *J Chromatogr B*. 805; 2004: 249-254.
- [10] Tang Y, He Y, Yao T and Zeng S, Stereoselective RP-HPLC determination of esmolol enantiomers in human plasma after precolumn derivatization, *J Biochem Biophys Methods*. 59; 2004:159-166.
- [11] Maurer H H, Tenberken O, Kratzsch C, Weber A A and Peters F.T., Screening for library-assisted identification and fully validated quantification of 22 beta-blockers in blood plasma by liquid chromatography-mass spectrometry with atmospheric pressure chemical ionization *J Chromatogr A*. 1058; 2004: 169-181.
- [12] Zuppa A F, Shi, Adamson H and Peter C., Liquid chromatography-electrospray mass spectrometry (LC-MS) method for determination of esmolol concentration in human plasma, *J Chromatogr B*. 796; 2003: 293-301...
- [13] Achari R, Drissel D and Hulse J D, Liquid-chromatographic analysis for esmolol and its major metabolite in urine, *Clin Chem*. 32; 1986: 374-376.
- [14] Malovaná S, Gajdošová D, Benedik J and Havel, J, Determination of esmolol in serum by capillary zone. electrophoresis and its monitoring in course of heart surgery, *J Chromatogr B*. 760; 2001: 37-43.

- [15] Vanita somasekhar and. D. Gowri sankar , Development and Validation of a Rapid RP-HPLC Method for the Estimation of Esmolol Hydrochloride in Bulk and Pharmaceutical Dosage Forms, E-Journal of Chemistry. 3; 2010: 807-812.
- [16] Liu, Zhong; Xu, Jun; Zhang, Yi; Chen, Guanrong, Determination of esmolol hydrochloride and related substances by HPLC Zhongguo Yiyuan Yaoxue Zazhi (2006), 26(1), 104-106.
- [17] Smita Sharma, M. C. Sharma*, D. V. Kohlib, A. D. Sharmac, Development and Validation of TLC-Densitometry Method for Simultaneous Quantification of Montelukast Sodium and Levocetirizine Dihydrochloride Pharmaceutical Solid dosage form Der, Pharmacia Lettre 2010: 2 (1) 489-494
- [18] Shanmukha, Kumar J. V.; Geeta, Swarupa P.; Vardhan, S. V. M.; Ramachandran, D, Spectrophotometric determination of montelukast sodium in bulk and pharmaceutical formulations, Pharma Chemica (2012), 4(2), 720-724.
- [19] Patel, Nilam K.; Pancholi, S. S., Spectrophotometric determination of montelukast sodium and levocetirizine dihydrochloride in tablet dosage form by AUC curve method, Pharma Chemica (2011), 3(5), 135-140
- [20] Bankar, Rima M.; Patel, Dipti B., Spectrophotometric determination of Montelukast Sodium and Desloratadine in combined dosage form, International Journal of ChemTech Research (2013), 5(1), 136-141.
- [21] Shi, Yun-feng; Lin, Li-qin, HPLC determination of montelukast sodium tablets and its related substances, Yaowu Fenxi Zazhi (2013), 33(2), 308-311.
- [22] Naga Raju, K.; Gopala Swamy, T.; Lakshmana Rao, A., Development and validation of RP-HPLC method for the determination of montelukast sodium in bulk and in pharmaceutical formulation, International Journal of Pharmaceutical, Chemical and Biological Sciences (2011), 1(1), 12-16
- [23] Shakalisava, Yuliya; Regan, Fiona, Determination of montelukast sodium by capillary electrophoresis, Journal of Separation Science (2008), 31(6-7), 1137-1143.
- [24] Alsarra, I.; Al-Omar, M.; Gadkariem, E. A.; Belal, F., Voltammetric determination of montelukast sodium in dosage forms and human plasma, Farmaco (2005), 60(6-7), 563-567.
- [25] J.J. Berzas Nevado, J. Rodr'iguez Flores*, G. Castan'eda Pen'algo, N. Rodr'iguez Farin'as, Determination of sildenafil citrate and its main metabolite by Sample stacking with polarity switching using micellar electrokinetic chromatography, Journal of Chromatography A, 953 (2002) 279-286
- [26] Cao, Ling; Wu, Yan-yan; Zhang, Yu-lin; Lu, Yin-sheng, Evaluation of uncertainty for determination of sildenafil citrate added in health products by HPLC, Xiandai Shipin Keji (2012), 28(7), 890-893.
- [27] Ghodsi, Razieh; Kobarfard, Farzad; Tabatabai, Sayyed Abbas, Application of narrow-bore HPLC columns in rapid determination of sildenafil citrate in its pharmaceutical dosage Forms, Iranian Journal of Pharmaceutical Research (2012), 11(1), 123-127.
- [28] Zhong, Hua; Liang, Shu-ming; Zeng, Wei-jie; Gao, Kai-wen, Determination of sildenafil citrate in health foods by high performance liquid chromatography with solid phase extraction, Xiandai Shipin Keji (2010), 26(2), 206-208.
- [29] Lopes, Antonio Carlos V., Jr.; Luz, Rita de Cassia Silva; Damos, Flavio S.; dos Santos, Alexandre S.; Franco, Diego L.; Pio dos Santos, Wallans T., Determination of sildenafil citrate (Viagra) in various pharmaceutical formulations by flow injection analysis with multiple pulse amperometric detection, Journal of the Brazilian Chemical Society (2012), 23(10), 1800-1806.
- [30] Khalil, Sabry; El-Naggar, A. Y., Analytical utility of atomic emission spectrometry for the determination of sildenafil citrate (Viagra) in pharmaceutical formulations, Analytical Chemistry: An Indian Journal (2012), 11(2), 66-72.
- [31] Frag, Eman Y. Z.; Mohamed, Gehad G.; Alelaiwi, Hana M. S. Utility of ion-associate formation reactions for the spectrophotometric determination of sildenafil citrate in pure form and in Virecta tablets, Pharmaceutica Analytica Acta (2011), 2(6), 131.
- [32] Thangabalan, B.; Vadivel, K.; Sowjanya, K.; Tejaswi, G.; Thejaroop, N.; Manohar babu, S.; Vijayaraj Kumar, P., Quantitative spectrophotometric determination of sildenafil citrate in tablet formulation using urea as hydrotropic solubilizing agent, Research Journal of Pharmaceutical, Biological and Chemical Sciences (2011), 2(2), 235-239
- [33] Ren, Shuling; Zhu, Xujiang, Determination of sildenafil citrate-illegally mixed into Jinsuo Gujing pills by LC-MS, Zhongguo Xiandai Yingyong Yaoxue (2010), 27(4), 358-360..
- [34] Suma, B. V.; Kannan, K.; Madhavan, V.; Nayar, Chandini R., HPTLC method for determination of Terbinafine in the bulk drug and tablet dosage form, International Journal of ChemTech Research (2011), 3(2), 742-748.
- [35] Faridbod, Farnoush; Ganjali, Mohammad Reza; Norouzi, Parviz, Potentiometric PVC membrane sensor for the determination of Terbinafine, International Journal of Electrochemical Science (2013), 8(5), 6107-6117.
- [36] Li, Yan-bo; Wang, Chao; Zhao, Xiao-dong; Lu, Jun; Chen, Xiao-hui, Determination of terbinafine hydrochloride content in functional cosmetics by high performance liquid chromatography, Riyong Huaxue Gongye (2012), 42(3), 234-236.
- [37] He, Wenyue; Xu, Airen; Chen, Wu; Ma, Weicheng, Determination of terbinafine in human plasma by RP-HPLC, Zhongguo Yaoshi (Wuhan, China) (2012), 15(9), 1276-1278.
- [38] Mrutyunjayarao, R.; Naresh, Saranapu; Pendem, Krishna; Rao, Psnh Ramachandra; Sasstry, C. S. P.; Viplava Prasad, U., Three Simple Spectrometric Determination of Terbinafine Hydrochloride (TRB) in Pure State and Tablets, Proceedings of the National Academy of Sciences, India, Section A: Physical Sciences (2012), 82(3), 221-224.
- [39] Chennaiah, M.; Veeraiah, T.; Kumar, T. Vinod; Venkateshwarlu, G., Extractive spectrophotometric methods for determination of terbinafine hydrochloride in pharmaceutical formulations using some acidic triphenylmethane dyes, Indian Journal of Chemical Technology (2012), 19(3), 218-221
- [40] Ramesh Sawant, Lokesh Bhangale, Rupali Joshi and Prashant Lanke, Validated spectrophotometric methods for simultaneous estimation of Paracetamol, Domperidone and Tramadol HCl in pure and tablet dosage form, J. Chem. Metrol. 4:1 (2010) 21-27
- [41] Vinay, Kanakapura B.; Revenasiddappa, Hosakere D.; Abdulrahman, Sameer A. M., Sensitive spectrophotometric determination of tramadol hydrochloride in pharmaceuticals using Folin-Ciocalteu's reagent, Turkish Journal of Pharmaceutical Sciences (2013), 10(1), 57-68.
- [42] Krishna, L. Mohan; Reddy, P. Jayachandra; Reddy, V. Jaya Sankar; Rao, K. V. S. Prasada, Spectrophotometric determination of tramadol hydrochloride using quinines, Journal of the Institution of Chemists (India) (2012), 84(4), 111-113.
- [43] Qu Lingbo; Feng Shuhui; Wu Yongjun; Wu Yuming, HPLC method for determination of tramadol hydrochloride in human plasma Sichuan da xue xue bao. Yi xue ban = Journal of Sichuan University. Medical science edition (2003), 34(3), 574-5,
- [44] Kucuk Aysel; Kadioglu Yucel, Determination of tramadol hydrochloride in ampoule dosage forms by using UV spectrophotometric and HPLC-DAD methods in methanol and water media, Farmaco (Societa chimica italiana : 1989) (2005), 60(2), 163-9
- [45] Abu Shawish, Hazem M.; Al-Dalou, Ayoub R.; Abu Ghalwa, Nasser; Abou Assi, Anwar A., Potentiometric sensor for determination of tramadol hydrochloride in pharmaceutical preparations and biological fluids, Pharmaceutica Analytica Acta (2010), 1(1), 103
- [46] Maha, El-Tohamy; Magda, El-Maamly; Waffa, S. Hassan; Shalaby, Abdalla, Direct potentiometric determination of tramadol hydrochloride in pharmaceutical preparations and biological fluids, Oriental Journal of Chemistry (2006), 22(3), 517-526

- [47] Huang, Chaolun; Dong, Qiuxiang; Ji, Guorong; Cai, Binxue; Wu, Haiyan, Determination of tramadol hydrochloride and its tablets by conductometric titration, *Zhongguo Yiyao Gongye Zazhi* (2006), 37(2), 115-117
- [48] Sara Abdulgader Mohammed Ebraheem, Abdalla Ahmed Elbashir, Spectrophotometric method for the determination of ofloxacin and levofloxacin in pharmaceutical formulations, *American Academic & Scholarly Research Journal* Vol. 4, No. 2, March 2012
- [49] B Narayana & K Ashwini, Spectrophotometric determination of frusemide by its oxidation with ceric ammonium sulphate, *Indian journal of chemical technology* vol.17, march-2010,pp 150-153

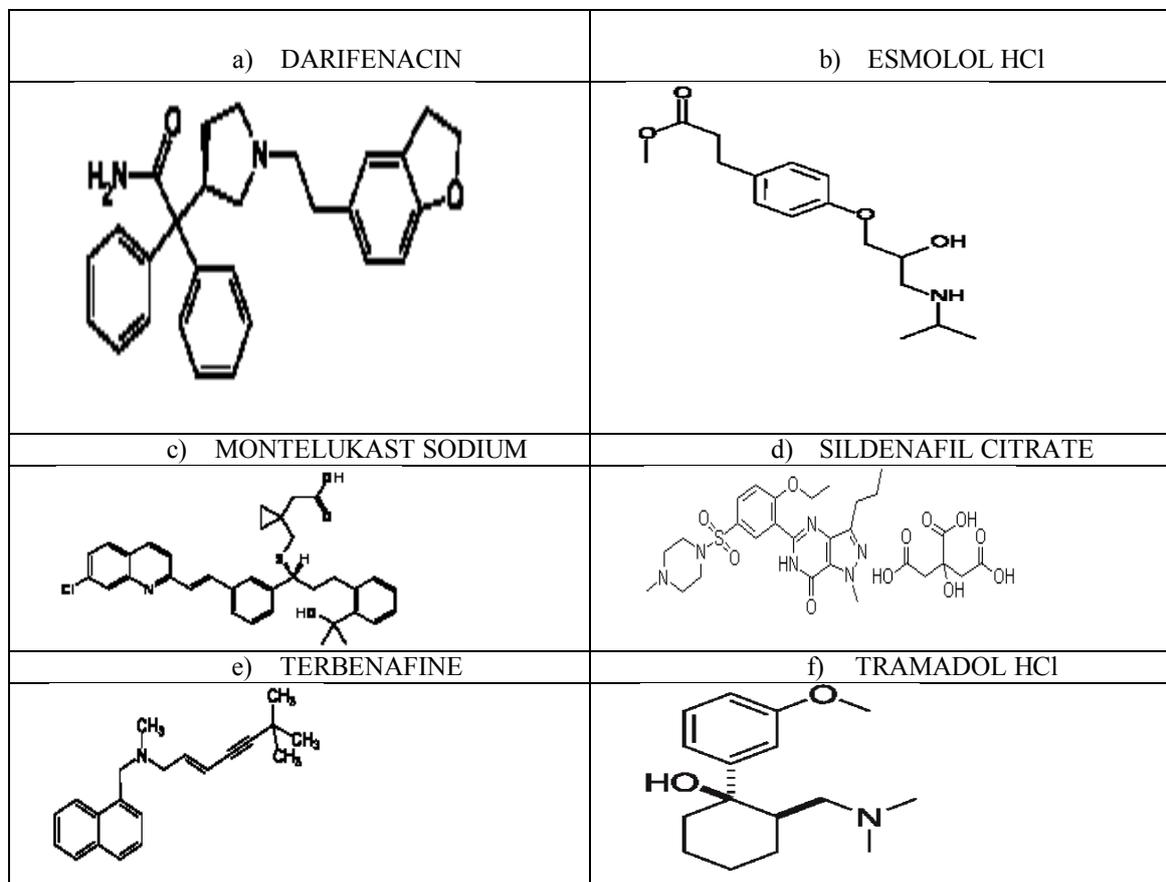
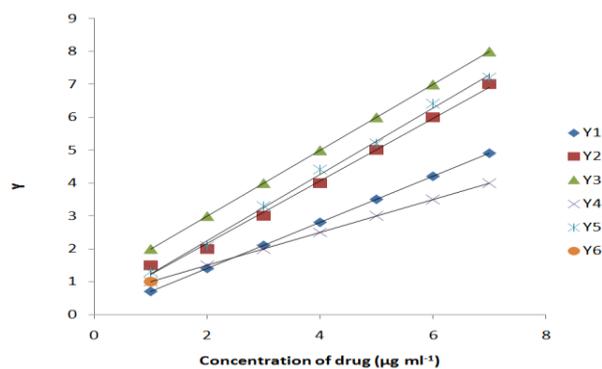


Fig.1: structures of drugs



$$Y = Y_1, Y_2, Y_3, Y_4, Y_5, Y_6$$

$$Y_1 = 1.4 + \text{Absorbance of DAR}$$

$$Y_2 = 2 + \text{Absorbance of ESM}$$

$$Y_3 = 2 + \text{Absorbance of MON}$$

$$Y_4 = 20 + \text{Absorbance of SIL}$$

$$Y_5 = 3 + \text{Absorbance of TER}$$

$$Y_6 = 2 + \text{Absorbance of TRA}$$

Fig.2: Calibration curves of the drugs

Table 1: Analytical and regression parameters of Spectrophotometric method

Parameter	DAR	ESM	MON	SIL	TER	TRAM
λ_{\max} (nm)	523	523	523	523	523	523
Beer's law limits ($\mu\text{g ml}^{-1}$)	0.7-7.0	2-14	1-7	5-35	3-21	2-14
Molar absorptivity ($\text{L mol}^{-1} \text{cm}^{-1}$)	3.7×10^6	2.5×10^5	3.5×10^6	4.3×10^5	1.07×10^6	2.3×10^6
Sandell sensitivity* ($\mu\text{g cm}^{-2}$)	0.0085	0.0018	0.1045	0.0926	0.0289	0.0085
Limit of detection ($\mu\text{g ml}^{-1}$)	0.3517	0.1796	0.5862	2.6889	2.0637	0.3517
Limit of quantification $\mu\text{g ml}^{-1}$	1.0656	0.5450	1.7763	81.480	6.2536	1.0656
Regression equation ($Y^{**}=a+bX$)	0.1808	0.0019	0.2324	0.2183	0.1199	0.1436
Intercept, (a)	0.0166	0.0093	0.041	0.0023	0.0158	0.0166
Slope, (b)	0.1173	0.5433	0.0957	0.0108	0.0347	0.1173
Correlation coefficient, (r)	0.9872	0.9882	0.9947	0.994	0.994	0.987
Standard deviation of intercept (S_a)	0.0125	0.0296	0.017	0.0088	0.0217	0.0125
Standard deviation of slope (S_b)	0.0050	0.0296	0.0048	0.0008	0.0020	0.0047

*Limit of determination as the weight in μg per mL of solution, which corresponds to an absorbance of $A = 0.001$ measured in a cuvette of cross-sectional area 1 cm^2 and path length of 1 cm . $Y^{**} = a+bX$, where Y is the absorbance and X =concentration of drug ($\mu\text{g ml}^{-1}$)

Table 2: Determination of accuracy and precision of the methods on pure drug Samples

Drug	Taken ($\mu\text{g ml}^{-1}$)	Found ($\mu\text{g ml}^{-1}$)	ER (%)	Recovery (%)	RSD(%)	Proposed method Mean \pm SD
DAR	1.5	1.49	0.67	99.33	0.4669	99.92 ± 0.4651
	2.5	2.49	0.40	99.60		
	3.0	3.01	0.33	100.30		
	4.5	4.52	0.44	100.44		
	5.5	5.5	0.00	100.00		
ESM	3.0	2.99	0.33	99.67	0.2482	99.96 ± 0.251
	5.0	5.01	0.20	100.2		
	7.0	6.98	0.29	99.71		
	9.0	9.01	0.11	100.11		
	11.0	11.02	0.18	100.1		
MON	1.5	1.49	0.67	99.33	0.5232	99.90 ± 0.504
	2.5	2.51	0.40	100.4		
	3.5	3.48	0.57	99.43		
	4.5	4.52	0.44	100.44		
	5.5	5.49	0.18	99.82		
SIL	11.0	11.02	0.18	100.18	0.9703	99.80 ± 0.965
	15.0	14.99	0.22	99.93		
	18.0	18.01	0.06	100.05		
	22.0	21.99	0.45	99.96		
	25.0	24.47	0.12	97.88		
TER	4.0	3.97	0.75	99.25	0.2454	99.55 ± 0.239
	5.0	4.98	0.40	99.60		
	7.0	6.99	0.14	99.86		
	8.0	7.98	0.25	99.75		
	10.0	9.98	0.20	99.80		
TRA	3.0	2.98	0.67	99.33	0.2889	99.82 ± 0.302
	5.0	4.99	0.20	99.80		
	7.0	7.01	0.14	100.14		
	9.0	8.98	0.22	99.78		
	11.0	10.97	0.27	99.73		

Table 3: Results of assay of tablets by the proposed methods and statistical evaluation and recovery experiments by standard addition method.

Pharmaceuticals/ tablets/ injection	Drug in tablet ($\mu\text{g ml}^{-1}$)	Drug added ($\mu\text{g ml}^{-1}$)	Total found ($\mu\text{g ml}^{-1}$)	ER%	Recovery %	RSD%	Reference method mean \pm SD	Proposed method \pm SD
DAR (VESIGARD)	1.0	0.5	1.52	1.33	101.33	0.864	99.69 \pm 0.909	100.16 \pm 0.863
	2.0	1.0	2.98	0.67	99.33			
	3.0	0.00	3.01	0.33	100.33			
	4.0	0.00	3.99	0.25	99.75			
ESM (CLOL)	1.0	0.5	1.49	0.67	99.33	0.940	99.98 \pm 0.93	100.18 \pm 0.938
	1.0	1.0	2.03	1.50	101.50			
	2.0	1.0	3.01	0.33	100.33			
	3.0	1.0	3.99	0.25	99.15			
MON (MONTAIR)	1.0	1.0	1.99	0.50	99.50	0.730	101.07 \pm 0.115	100 \pm 0.725
	1.0	2.0	3.02	0.67	100.67			
	2.0	0.00	1.98	1.00	99.00			
	3.0	0.00	2.98	0.67	99.33			
SIL (ALISIGA)	10	0.5	10.48	0.19	99.80	0.120	99.80 \pm 0.90	100.10 \pm 0.995
	20	2.0	22.02	0.09	100.09			
	20	1.0	20.98	0.10	99.90			
	30	0.00	29.98	0.06	99.93			
TER (TEBINA)	4.0	1.00	4.97	0.60	99.40	0.324	100.27 \pm 0.58	99.65 \pm 0.315
	5.0	1.00	6.01	0.17	100.17			
	7.0	0.00	6.98	0.28	99.71			
	8.0	0.00	7.97	0.38	99.63			
TRA (DOLOTRAM)	3.0	1.00	4.02	0.50	100.50	0.394	101.20 \pm 0.3558	99.89 \pm 0.401
	5.0	0.5	5.48	0.36	99.64			
	7.0	0.5	7.48	0.27	99.73			
	9.0	1.0	9.98	0.20	99.80			

Table 4: Student's t-test and f-test values for pharmaceutical analysis

Pharmaceuticals/ tablets/injection	DAR (VESIGARD)	ESM (CLOL)	MON (MONTAIR)	SIL (ALISIGA)	TER (TEBINA)	TRA (DOLOTRAM)
Student's t-test	0.081 (2.51)	0.019 (2.57)	0.663 (2.23)	0.137 (1.27)	0.599 (1.735)	0.104 (0.18)
f-test	0.902 (4.21)	1.016 (4.95)	0.397 (5.67)	1.222 (3.56)	0.295 (2.246)	1.266 (1.65)