A Validated Reversed Phase HPLC Method for Simultaneous Determination of the antihistaminic Cetirizine and Beta2-adrenergic agonist Salbutamol in their Co-formulated Tablets

Fatma Ahmed Aly, Nahed EL-Enany, Heba Elmansi* and Amany Nabil
Department of Analytical Chemistry, Faculty of Pharmacy, Mansoura University, Egypt

Abstract

A new HPLC method is adopted in this research for simultaneous determination of Cetirizine (CTZ) and Salbutamol (SAL) in their tablets. The developed method used C18 column and a mobile phase composed of methanol: 0.1M phosphate buffer in the ratio (80:20 v/v) operating at pH 3.5. Nimesulide was used as internal standard (IS). The peak area ratio-concentration plot indicated the linearity over ranges of 5-50 and 4-80 µg/mL for CTZ and SAL with limit of detection of 0.57 and 2.00 µg/mL respectively. Comparing the proposed method with a comparison method revealed that there was no significant difference between the two methods in regards to accuracy and precision.

Introduction

Cetirizine (CTZ) and Salbutamol (SAL) are co-formulated for treatment of common cold and allergy [1]. Cetirizine (CTZ, Figure 1a) is 2-[4-[4-(Chlorophenyl) phenylmethyl]-1–piperazinyl] ethoxy] acetic acid. It is long acting antihistaminic which used for relief of allergic conditions [2]. The BP described non-aqueous potentiometric titration method for CTZ determination [3]. Previously published methods described for determinations of CTZ include HPLC either in pharmaceutical preparations [4-6] or in biological fluids [7,8], fluorimetry [9] and spectrophotometry [10].

Salbutamol (SAL, Figure 1b) has a chemical name of 2-tert-Butylamino-1-(4-hydroxy-3-hydroxymethylphenyl) ethanol. It is B2-receptor agonist which acquiring a bronchodilation action and used for the treatment of chronic obstructive pulmonary disease and in managing asthma [2]. The BP recommended non-aqueous potentiometric titration method using perchloric acid as a titrant [3]. Determination of salbutamol was reported in several former publications such as HPLC [11-13], spectrophotometry [14,15], flow injection [16,17], capillary electrophoresis [18].

The combination of CTZ and SAL with a pharmaceutical ratio of 5:2 respectively is present under the trade name Vetirex1. This combination is used for common cold and allergic conditions. Up till now; no reported method was described for the determination of CTZ and SAL. This encourages us to develop a simple HPLC method for their simultaneous determination.

Experimental

HPLC analysis was performed using Perkin Elmer TM series 200 chromatograph (USA) with 200 µL loop supplied with injector valve of Rheodyne. Series 200 UV/VIS. Detector was used and set at wave length 230 nm. The column used for analysis was Shimadzu VP-ODS column (250 mm x 4.6 mm i.d., 5 µm particle size).

Materials and reagents

Cetirizine (batch # 3003CZ8RJ) pure sample was provided from Apex Co. (Cairo, Egypt). Its percentage purity was found to be 99.95(as labeled). Salbutamol (batch # 511/55/03/5018) was obtained from Pharaonia Co. Alex, Egypt. Its purity was found to be 100.15% as labeled from the manufacturer. Nimesulide (IS) was of 99.90% purity, with batch No# 000604. It is provided from Pharaonia Co., Alex, Egypt. Zyrttec® tablet (batch #6221045001829) labeled to contain 10 mg CTZ/tablet, manufactured by Glaxosmithkline Company obtained from a community pharmacy. Ventolin® (2mg) tablet (batch #6221045000969) contains 2 mg SAL per tablet, produced by Glaxosmithkline Company bought from a community pharmacy. Sodium dihydrogen phosphate and sodium hydroxide were provided from ADWIC CO., Egypt. Solvents (HPLC grade) were obtained from Sigma-Aldrich (Germany). O-phosphoric acid (85 % w/v) was provided from Riedel-deHaen (Germany).

Keywords: CTZ: Cetirizine; co-formulated tablet; HPLC; SAL: Salbutamol
Optimum chromatographic conditions

Shimadzu VP-ODS column (250 mm x 4.6 mm i.d., 5 µm particle size), Japan was used as a chromatographic column. The separation was carried out at room temperature at 1 mL/min flow rate. The mobile phase composed of methanol: 0.1M sodium dihydrogen phosphate (80:20 v/v). PH was justified to 3.5. The detector wavelength was set at 230nm.

Standard solutions

Accurately weighed 20 mg of CTZ, SAL and IS drug was dissolved in 100 mL of methanol to obtain stock solutions of 200 µg/mL.

General procedures

Construction of calibration graphs

Aliquot volumes of a stock solution of CTZ and SAL were taken into a set of 10 mL volumetric flask. The solutions were diluted to the volume with the mobile phase at pH 3.5 to obtain a final concentration of 5-50 and 4-80 µg/mL for CTZ and SAL, respectively. NIM (IS) in 20 µg/mL concentration was added. Plotting peak area ratio versus the concentration in µg/mL gave us the corresponding calibration graphs.

Analysis of laboratory- prepared mixture of CTZ and SAL

Laboratory prepared mixture of CTZ and SAL were freshly prepared by mixing different volumes of the drug in the ratio5:2, respectively together with the IS and then the procedure for chromatographic analysis under optimum conditions were performed. The mean percent recoveries of each drug were determined from the previously plotted calibration curve.

Analysis of laboratory- prepared co-formulated tablets

Aliquots of the accurately weighed powdered tablets equivalent to 5.0 mg CTZ and 2.0 mg SAL were transferred to volumetric flasks 100 mL volume. Nearly 80 mL methanol was added and undergoes sonication for 10 minutes. The volume was then completed with methanol and filtered. Aliquots of this solution with the IS were transferred to a series of 10 mL volumetric flasks to obtain suitable concentrations within the working range. The mobile phase containing the mixture was eluted under suitable chromatographic conditions. The mean percent content was calculated using the regression equations or calibration graphs.

Analysis of single ingredient tablets of each drug

Ten tablets of Zyrtec® for CTZ or Ventolin® in case of SAL have pulverized accurately and mixed well. An accurately weighed quantity of 20 mg of each drug was transferred to 100 mL volumetric flasks, 80 mL methanol was added and sonicated for 10 min. Solutions were completed with methanol to the volume then filtered. A further dilution was performed to obtain the working concentration range after adding IS. The mean percent content was calculated.

Results and Discussion

The present study describes a simple and reliable HPLC method for the simultaneous determination of CTZ and SAL in their co-formulated tablets. After optimization of the chromatographic conditions, CTZ was well separated from SAL with in short retention time (less than 5 min), with a higher number of theoretical plates at pH 3.5 with UV detection at 230 nm (Figures 2 and 3).

Optimization of chromatographic conditions

Typical chromatogram of CTZ and SAL is illustrated in Figure 2. To achieve good separation in short run time, chromatographic conditions were optimized. The studied compounds exhibited
maxima in their spectra at 211 and 231 nm for CTZ and 228 nm and 278 nm for SAL. Therefore, the UV detection wavelength was selected to be 230 nm that allowed simultaneous determination of the two drugs with suitable sensitivity (Figure 4).

Different columns were tried to choose the most suitable one for separation of the two drugs, this includes Shimadzu VP-ODS C18 column (250 mm), Shimadzu VP-ODS C18 column (150 mm) and Shim-pack CLC C8 column (250 mm). The first column allowed peaks separations with good resolution while the second and the third columns yielded overlapped peaks.

To enhance resolution, efficiency and for good separation, modifications were made in the mobile phase composition including the organic modifier type and ratio, mobile phase pH and ionic strength of the buffer, the results were further explained in Table 1.

**Table 1: Optimization of chromatographic conditions for HPLC determination of the studied drugs.**

| Parameter               | CTZ | SAL  | Resolution (R<sub>t</sub>) | Tailing factor (T) | Capacity Factor (K') | Selectivity factor (α) |
|-------------------------|-----|------|-----------------------------|-------------------|----------------------|------------------------|
| pH of the mobile phase  | 2.5 | 1156 | 829                         | 4.3               | 1                    | 1.25                   | 3.1            | 0.24 | 13    |
|                         | 3.5 | 2600 | 1225                        | 5.006             | 1                    | 1.13                   | 6.6            | 1.5  | 4.4   |
|                         | 4   | 1296 | 1056                        | 4.98              | 1.3                  | 1.5                    | 1.17           | 0.22 | 5.32  |
|                         | 5   | 1032 | 987                         | 3.98              | 1.31                 | 1.51                   | 2.1            | 0.6  | 3.4   |
| Conc. of phosphate buffer | 0.05 | 1600 | 1089                        | 5.423             | 1.02                 | 1.2                    | 3              | 0.1  | 30    |
|                         | 0.1 | 2600 | 1225                        | 5.006             | 1                    | 1.13                   | 6.6            | 1.5  | 4.4   |
|                         | 0.2 | 2381 | 1225                        | 6.2               | 1.25                 | 1.04                   | 3.36           | 0.25 | 13.4  |
| Conc. of methanol (%v/v)| 50% | 792  | 1024                        | 6.3               | 2.3                  | 2.49                   | 1.2            | 0.13 | 9     |
|                         | 70% | 1995 | 924                         | 5.568             | 1.7                  | 1.25                   | 3.1            | 0.24 | 13    |
|                         | 80% | 2600 | 1225                        | 5.006             | 1                    | 1.13                   | 6.6            | 1.5  | 4.4   |
|                         | 90% | 797  | 1344                        | 2.795             | 1.14                 | 0.99                   | 1.5            | 0.5  | 3     |
| Type of organic modifier| propanol | 1251 | 984 | 3.007 | 2.52 | 1.28 | 0.67 | 0.19 | 3.46 |
|                         | acetone | 1212 | 1175 | 1.298 | 1.54 | 1.53 | 0.45 | 0.31 | 1.45 |
|                         | methanol | 2600 | 1225 | 5.006 | 1    | 1.13 | 6.6  | 1.5  | 4.4   |
| Flow rate (mL/min)     | 0.8  | 1394 | 1024 | 4.79  | 1.31 | 1.61 | 1    | 0.14 | 7.05  |
|                         | 1    | 2600 | 1225 | 5.006 | 1    | 1.13 | 6.6  | 1.5  | 4.4   |
|                         | 1.2  | 1277 | 887  | 2.309 | 1.07 | 1.25 | 1    | 0.15 | 6.6   |

**Number of theoretical plates (N) = \( \frac{5.54(\frac{R}{t})^2}{\text{R}_{2 \text{m}}} \)**

**Resolution (R<sub>t</sub>) = \( \frac{\text{R}_{1 \text{m}} - \text{R}_{2 \text{m}}}{\text{R}_{2 \text{m}}} \)**

**Tailing factor (T) = \( \frac{W_{R1}}{2t} \)**

**Selectivity factor (Relative retention) (α) = \( \frac{t_{R1}}{t_{R2}} \)**

**Capacity factor (K') = \( \frac{t_{R1} - t_{R2}}{t_{R2}} \)**
To determine the suitable organic modifier, methanol, acetoniitrile and n-propanol were used. It was found that acetoniitrile and n-propanol showed overlapping peaks of the two drugs. Methanol was chosen for well separated peaks with an increased number of theoretical plates.

The result of altering mobile phase composition was checked using different mobile phases in which methanol ratio varies from 50 to 90% v/v. It was found that ratio 50-70% v/v methanol showed broad CTZ peak. Ratio more than 80% v/v methanol resulted in overlapped peaks. The ratio of (80:20 v/v) methanol: phosphate buffer resulted in well-separated peaks within a reasonable resolution time and with the higher theoretical plates.

The effect of pH of the mobile phase was studied over the range of 2.5-5.5. The peak area ratio of CTZ and SAL decreased with pH higher than 3.5 accompanied by decreasing in the number of theoretical plates. At pH lower than 3.5 there were overlapping between the solvent front and SAL peak. So, pH 3.5 was found to be the most suitable one.

The influence of ionic strength of phosphate buffer was investigated with mobile phases containing 0.05- 0.2 M phosphate buffer. 0.1 M phosphate buffer was selected as it gave well resolved peaks with a higher number of theoretical plates.

Drotroverin, ketoconazole guaifenesin, ambroxol metoclopramide, and nimesulide were tested for the choice of IS.

### Table 2: Analytical data of CTZ and SAL determination by the proposed method.

| Parameter                        | HPLC Method |                |                |
|----------------------------------|-------------|----------------|----------------|
|                                  | CTZ         | SAL            |                |
| Linearity range (µg/mL)          | 5.0-50.0    | 4.0-80.0       |                |
| Intercept (a)                    | -0.081      | 0.192          |                |
| Slope (b)                        | 0.035       | 0.01           |                |
| Correlation coefficient (r)      | 0.9999      | 0.9999         |                |
| S.D. of residuals (S_x)          | 6.633 x 10^{-2} | 6.66 x 10^{-2} |                |
| S.D. of intercept (S_y)          | 4.515 x 10^{-3} | 4.866 x 10^{-3} |                |
| S.D. of slope (S_b)              | 1.707 x 10^{-4} | 1.013 x 10^{-4} |                |
| S.D.                             | 1.64        | 0.35           |                |
| % RSD                           | 1.64        | 0.36           |                |
| % Error                         | 0.67        | 0.58           |                |
| LOD (µg/mL)                      | 0.57        | 2.004          |                |
| LOQ (µg/mL)                      | 1.9         | 4.88           |                |

* Percentage relative standard deviation.

### Table 3: Application of the proposed and comparison methods [4,11] for determination of the studied drugs.

| Compound | HPLC Method | comparison methods [4,11] |
|----------|-------------|--------------------------|
|          | Amount taken (µg/mL) | Amount found (µg/mL) | % Found | Amount taken | Amount found | % Found |
| CTZ      | 5            | 5.097                    | 101.94 | 5            | 4.98        | 99.58   |
|          | 7            | 7.11                     | 101.63 | 7            | 7.04        | 100.59  |
|          | 10           | 9.83                     | 98.29  | 9            | 8.98        | 99.77   |
|          | 25           | 24.89                    | 99.54  | 9            | 8.98        | 99.77   |
|          | 30           | 29.67                    | 98.89  | 9            | 8.98        | 99.77   |
|          | 50           | 50.03                    | 100.06 | 9            | 8.98        | 99.77   |
| Mean     | 100.06       | 99.98                    |        |              |             |        |
| ± S.D.   | 1.64         | 0.58                      |        |              |             |        |
| t        | 0.087        |                           |        |              |             |        |
| F        | 7.5          |                           |        |              |             |        |
| SAL      | 4            | 3.9                      | 97.5   | 10           | 10.1        | 101.01  |
|          | 10           | 9.8                      | 98     | 20           | 19.79       | 98.99   |
|          | 35           | 35.1                     | 100.29 | 30           | 83.01       | 100.34  |
|          | 50           | 49.5                     | 99     | 99           |             |        |
|          | 60           | 60.9                     | 101.5  | 100.11       |             |        |
|          | 80           | 79.1                     | 98.9   | 100.11       |             |        |
| Mean     | 99.2         |                           |        | 100.11       |             |        |
| ± S.D.   | 0.35         |                           |        | 1.437        |             |        |
| t        | 0.95         |                           |        |              |             |        |
| F        | 2.07         |                           |        |              |             |        |

**N.B.** Each result is the average of three separate determinations.

The value of tabulated t and F are 2.20 and 19.29, respectively at P = 0.05."
Table 4: Precision data of the proposed method.

| Parameters | Intra-day                     | Inter-day                    |
|------------|------------------------------|------------------------------|
|            | x ± S.D | % RSD | % Error | x ± S.D | % RSD | % Error |
| SAL (µg/mL)|          |       |         |         |       |         |
| 10.0       | 98.95 ± 0.51 | 0.51 | 0.30 | 99.68 ± 1.06 | 1.06 | 0.61 |
| 20.0       | 99.05 ± 0.42 | 1.44 | 0.83 | 99.57 ± 0.59 | 0.59 | 0.34 |
| 50.0       | 98.30 ± 0.62 | 0.64 | 0.37 | 99.92 ± 0.39 | 0.39 | 0.23 |
| CTZ (µg/mL)|          |       |         |         |       |         |
| 25.0       | 98.20 ± 0.30 | 0.31 | 0.18 | 99.6 ± 1.08 | 1.09 | 0.63 |
| 30.0       | 97.87 ± 0.81 | 0.83 | 0.48 | 100.1 ± 0.90 | 0.90 | 0.52 |
| 50.0       | 98.57 ± 1.48 | 1.50 | 0.86 | 98.38 ± 0.82 | 0.84 | 0.48 |

N. B. Each result is the average of three separate determinations.

Table 5: Application of the proposed and comparison methods for determination of the studied drugs in different laboratory prepared mixtures in different pharmaceutical ratios.

| Parameter | Proposed method | Comparison methods*11 |
|-----------|-----------------|------------------------|
|           | Amount taken (mg/mL) | Amount found (mg/mL) | % Found | Amount taken (mg/mL) | % Found |
| CTZ       | 10 4             | 9.917                | 3.947   | 99.17 | 98.68 | 6.25 | 2.5 | 101.54 | 100.56 |
| SAL       | 25 10            | 25                   | 10.105  | 100   | 101.05 | 7.5  | 3   | 100.51 | 100.63 |
| HPLC      |                 |                       |         |       |       |       |     |     |       |
| Method    | 30 12            | 29.667               | 11.947  | 98.89 | 99.56 | 8.75 | 3.5 | 100.22 | 99.6 |
|           | 20 20            | 19.917               | 20      | 99.59 | 100   | 9    | 9   | 100   | 98.39 |
|           | 15 30            | 14.833               | 29.95   | 98.89 | 99.82 | 5    | 10  | 99.24 | 101.27 |
| Mean      |                 |                       |         | 99.31 | 99.82 | 100.3 | 100.09 |
| ± S.D.    |                 |                       |         | 0.59  | 1.68  | 0.73 | 1.12 |
| t         |                 |                       |         | 0.708 | 1.224 |
| F         |                 |                       |         | 1.82  | 10.78 |

N. B. Each result is the average of three separate determinations.

The value of tabulated t and F are 2.13 and 6.40, respectively at P = 0.05%.

Citation: Aly FA, EL-Enany N, Elmansi H and Nabil A. A Validated Reversed Phase HPLC Method for Simultaneous Determination of the antihistaminic Cetirizine and Beta2-adrenergic agonist Salbutamol in their Co-formulated Tablets. SM Anal Bioanal Technique. 2017; 2(2): 1011.
The comparison method for CTZ [4] determination in bulk and formulation involved the use of HPLC technique using methanol: 0.01 M disodium hydrogen phosphate buffer (60: 40 v/v), with C18 column and UV detection at 217 nm. An HPLC method [11] was used as a comparison method for SAL determination using acetonitrile: phosphate buffer (65:35 v/v) as a mobile phase. Column C18 was used at 235 nm. The results were compared in terms of accuracy and precision using Student t-test and the variance ratio F-test [20]. No significant difference was observed (Table 3).

Intraday precision and Inter-day precision analyses were determined using the proposed procedures and as shown in Table 4.

The ability of the method to determine CTZ and SAL in their pharmaceutical co-formulated tablet without the interference of additives showed the selectivity of this method (Figure 3).

### Table 6: Application of the proposed and comparison methods for determination of the studied drugs in their laboratory prepared co-formulated tablets.

| Parameter | Proposed method | Comparison methods \[4,11\] |
|-----------|-----------------|--------------------------|
|           | Amount taken (mg/mL) | Amount found (mg/mL) | % Found | Amount taken (mg/mL) | Amount found (mg/mL) | % Found |
| HPLC Method | CTZ | SAL | CTZ | SAL | CTZ | SAL | CTZ | SAL |
| CTZ      | 10  | 4    | 10.17 | 3.962 | 101.7 | 99.05 | 6.25 | 2.5     |
| SAL      | 25  | 10   | 24.92 | 19  | 99.67 | 100  | 7.5 | 3       |
| Mean     | 30  | 12   | 30.5 | 11.89 | 101.7 | 99.04 | 8.75 | 3.5     |
| ± S.D.   | 1.41| 0.55 | 2.52 | 0.71 |
| t        | 0.75| 1.5  |
| F        | 2.87| 1.65 |

**N.B.** Each result is the average of three separate determinations.

The value of tabulated t and F are 2.76 and 19.00, respectively at P = 0.05\[20\].

### Table 7: Application of the proposed and comparison methods for determination of the studied drugs in their single ingredient commercial tablets.

| Dosage Form | HPLC Method | Comparison methods \[4,11\] |
|-------------|-------------|--------------------------|
|             | Amount taken (µg/mL) | Amount found (µg/mL) | % Found | Amount found (µg/mL) | % Found |
| Zyrtec\(^\text{®}\) tablets 10mg | 6  | 6.023 | 100.38 | 5.892 | 99.10 |
| CTZ/tablet  | 8  | 7.955 | 99.44 | 7.108 | 98.65 |
| Mean        | 10 | 10.023 | 100.23 | 9.98 | 100.00 |
| ± S.D.      |    | 0.51 | 1.56 |
| t           |    | 1.56 | 1.86 |
| F           |    | 1.45 | 9.78 |
| Valtolin\(^\text{®}\) tablets 2 mg | 10 | 10.105 | 101.05 | 10.082 | 100.82 |
| SAL/tablet  | 20 | 20.105 | 100.53 | 19.837 | 99.19 |
| Mean        | 30 | 30.263 | 100.88 | 30.082 | 100.27 |
| ± S.D.      |    | 0.37 | 1.15 |
| t           |    | 1.45 | 9.78 |
| F           |    | 1.45 | 9.78 |

**N.B.** Each result is the average of three separate determinations.

The value of tabulated t and F are 2.76 and 19.00, respectively at P = 0.05\[20\].

Applications

**Laboratory prepared mixture analysis**

Laboratory prepared mixture of CTZ and SAL was analyzed using the proposed method (Figure 2) and the results were compared statistically with those of comparison methods [4,11] as indicated in Table 5.

**Dosage form analysis**

The suggested procedures were additionally utilized for the determination of CTZ and SAL in their laboratory prepared binary and single ingredient commercial tablets without interference from the excipients. Statistical comparison of the results with that obtained from the comparison methods4,11 using Student t-test and variance ratio F- test20 revealed a non-significant difference in terms of accuracy and precision (Tables 6 and 7).
Conclusion

Our target was to develop new HPLC method for simultaneous determination of CTZ and SAL in their ratio (2.5:1). The suggested method was found to achieve simplicity, accuracy, and time-saving. It was applied successfully for determination of CTZ and SAL in their laboratory prepared co-formulated tablets in short analysis time (less than 5 min).

References

1. GenericPedia – Encyclopedia of Generic Drugs. 2016.
2. Sweetman SC. Martindale: The complete drug reference 36th edn, Pharmaceutical press, London, 2009.
3. British Pharmacopoeia version17, The Stationary Office: London; electronic version. Volumel&I,2013.
4. Suryan AL, Bhusari VK, Rasal KS, Dhaneshwar SR. Simultaneous Quantitation and Validation of Paracetamol, Phenypropanolamine Hydrochloride and Cetirizine Hydrochloride by RP-HPLC in Bulk Drug and Formulation. IntJPharmSoland Drug Res. 2011; 3: 303-308.
5. El Waliliya AFM, Koranya MA, El Gindyb A, Bedairc MF. Spectrophotometric and high performance liquid chromatographic determination of cetirizine dihydrochloride in pharmaceutical tablets. J Pharm and Biomed Anal. 1998; 17: 435-442.
6. Jabera AMY, Al Sherifeb HA, Al Omarib MM, Badwanb AA. Determination of cetirizine dihydrochloride, related impurities and preservatives in oral solution and tablet dosage forms using HPLC. J Pharm and Biomed Anal. 2004; 36: 341-350.
7. Rosseel MT, Lefebvre RA. Determination of cetirizine in human urine by high-performance liquid chromatography. J Chrom B: BiomedSci and Appl. 1991; 565: 504-510.
8. Macek J, Płaček P, Klima J. Determination of cetirizine in human plasma by high-performance liquid chromatography. J Chrom B: BiomedSci and Appl. 1999; 736:231-235.
9. Wei XL, Lei XR, Gong Q, Wang LS and Liao Y. Determination of Cetirizine Dihydrochloride by Anti-Fluorescence Quenching on Rhodamine B-Sodium Tetraphenylborate System, Guangpu xueyu guangpu fen xi = Guangpu. 2011; 31:1596-600.
10. Pourghazi K, Khoshhesab ZM, Golpayeganizadeh A, Shapouri MR, Aftrozi H. Spectrophotometric determination of cetirizine and montelukast in prepared formulations. Intern J Pharm and Pharm Sci. 2011; 3:128-130.
11. Madhani M, Singh R. Development and Validation of a Stability-Indicating HPLC Method for the Simultaneous Determination of Salbutamol Sulphate and Theophylline in Pharmaceutical Dosage Forms. J Anal and Bioanal Tech. 2011; 2:1-5.
12. Pai PNS, Rao GK, Murthy MS, Agarwal A, Puranik S. Simultaneous Determination of Salbutamol Sulphate and Bromhexine Hydrochloride in Tablets by Reverse Phase Liquid Chromatography. Indian j pharm sci. 2009; 71: 53-55.
13. Hutchings MJ, Paul JD, Morgan DJ. Determination of salbutamol in plasma by high-performance liquid chromatography with fluorescence detection. JChrom B: Biomed Sci and Appl. 1983; 277: 423-426.
14. Hadi H. Developed spectrophotometric determination of salbutamol sulphate in pharmaceutical samples by coupling with O-nitroaniline. Iraqi J Sci. 2008; 49: 12-17.
15. Mohamed GG, Khalil SM, Zayed MA, El-Shall MA, 2, 6-Dichloroquinone chloride and 7,7,8,8-tetracyanoquinodimethane reagents for the spectrophotometric determination of salbutamol in pure and dosage form. J Pharm and Biomed Anal. 2002; 28: 1127-1133.
16. Barnett NM, Hindson BJ, Lewis SW. Determination of Ranitidine and Salbutamol by Flow Injection Analysis with Chemiluminescence Detection. Anal ChimActa. 1999; 384: 151-158.
17. Šatinský D, Kariček R, Svoboda A. Using on-line solid phase extraction for flow-injection spectrophotometric determination of salbutamol. Anal ChimActa. 2002; 455: 103-109.
18. Felix FS, Quintino MSM, Carvalho AZ, Coelho LHG, do Lago CL, Angnes L. Determination of salbutamol in syrups by capillary electrophoresis with contactless conductivity detection (CE-C4D). J Pharm and BiomAnal. 2006; 40: 1288-1292.
19. ICH Harmonized Tripartite Guidelines, Validation of Analytical Procedures: Text and Methodology, Q2(R1), Current Step 4 Version, Parent Guidelines on Methodology. 6; 1996. Incorporated in November (2005).
20. Miller, JC. Statistics and Chemometrics for Analytical Chemistry, 5th edition, Harlow, Pearson Education Limited. 2005; 256: 39-73, 107-149.