Simultaneous Estimation of Irbesartan, Telmisartan, Hydrochlorothiazide and Ramipril in Combined Dosage forms by Validated HPTLC Method

Abstract

A sensitive, specific and precise high performance thin layer chromatographic method for estimation of Irbesartan (IRB), Telmisartan (TEL), Hydrochlorothiazide (HCZ) and Ramipril (RAM) has been developed and validated. The method employed TLC aluminium plates pre-coated with silicagel 60 F254 as the stationary phase. The solvent system consisted of acetonitrile: toluene: methanol: formic acid (8:10:2:0.6 v/v/v/v). Densitometric analysis of drugs was carried out in the absorbance mode at 210nm. The method was validated with respect to linearity, specificity, precision, limit of detection, limit of quantification and recovery. The linear regression analysis data for the calibration plots showed a good linear relationship with R^2 of 0.999 for all the drugs in the concentration range of 250-1250ng/spot for TEL and RAM and 500-2500ng/spot for IRB and HCZ respectively. Recovery of all drugs was achieved in the range of 98.85%-101.31% by developed method. Statistical analysis proves that the method is repeatable and specific for the estimation of all four drugs. Developed method was successfully applied for estimation of all four drugs in their combined dosage form.

Keywords: Irbesartan; Telmisartan; Hydrochlorothiazide; Ramipril; HPTLC; Simultaneous Estimation

Abbreviations: HPTLC: High Performance Thin Layer Chromatography; ng: Nanogram; R^2: Correlation Coefficient; TEL: Telmisartan; HCZ: Hydrochlorothiazide; RAM: Ramipril; IRB: Irbesartan; mg: Milligram; SD: Standard Deviation; RSD: Relative Standard Deviation; SE: Standard Error of Mean; %: Percentage

Introduction

For the determination of Irbesartan (IRB), Telmisartan (TEL), Hydrochlorothiazide (HCZ) and Ramipril (RAM), different analytical methods such as spectrophotometry [1-6], spectrophotometry [7,8], high Performance Liquid Chromatography (HPLC) [15-35], LC-MS [36-41], capillary zone electrophoresis [42] and High performance thin layer chromatography (HPTLC) [43-47] were reported individually or in combination with other drugs. But no method was reported so far for the simultaneous analysis of these drugs in ternary mixture. No HPTLC method was reported for simultaneous estimation of these drugs in combined dosage form. Therefore it was thought of interest to develop and validate HPTLC method for simultaneous estimation of IRB, TEL, HCZ and RAM in their combined dosage form. The present work deals with estimation of two of these two drugs in combined dosage form by HPTLC method.

Materials and Methods

Instrumentation

The HPTLC system (CAMAG, Switzerland) consisting of Linomat V semiautomatic spotting device, TLC Scanner IV (Camag, Muttenz, Switzerland), twin trough developing chamber (20×10cm), UV cabinet with dual wavelength lamps, winCATS software, syringe (100µl capacity, Hamilton) were used for chromatographic study.

Chemicals and reagents

IRB, TEL, HCZ and RAM were kindly supplied as gift samples by Madras Pharmaceuticals, Chennai. All chemicals and reagents used were of LR grade and purchased from S.D. Fine Chem Limited, Mumbai, India. Formulations containing IRB and HCZ, TEL and RAM were procured from local pharmacy.

Chromatographic conditions

Analysis was performed on 10×10cm aluminium plates pre-coated with 0.2mm layers of silica gel 60 F254 (Merck, Mumbai). Before use plates were washed with methanol, activated in an oven at 105°C for 20 min, then left to cool at room temperature. Standard solutions were applied to pre-washed activated plates, as 6mm bands, 6 mm apart, under a stream of nitrogen, by means of a Camag (Muttenz, Switzerland) Linomat V automated spray-on band applicator equipped with a Hamilton 100µl syringe (Reno, Nevada, USA). The plates were developed with 10ml of mobile phase, in a Camag twin trough chamber previously saturated with mobile phase vapour for 20 min.

After development the plates were removed from the chamber and dried in air. Densitometry was performed in reflectance mode with Camag TLC scanner 3 using WinCATS (version 1.4.4) software incorporating track position optimization. The slit
Simultaneous Estimation of Irbesartan, Telmisartan, Hydrochlorothiazide and Ramipril in Combined Dosage forms by Validated HPTLC Method

Preparation of stock solutions of IRB, HCZ, TEL and RAM

A standard stock solution was prepared by adding 10mg of IRB, TEL and RAM and 20mg of HCZ to a 10 ml volumetric flask. The volume was made up to the mark with methanol. The stock solution containing 1mg/ml of each of IRB, TEL and RAM and 2mg of HCZ drug was then stored in refrigerator. The working solutions were freshly prepared by diluting 2.5ml of the stock solution to 10ml with methanol.

Calibration curve preparation

Amount of standard solution equivalent to 0.25 to 1.25µg of TEL, IRB and RAM and 0.5 to 2.5µg of HCZ were applied to the TLC plate, developed, dried and scanned as mentioned above. This was done by spotting 1,2,3,4 and 5µl of working solution (0.5µg/ml of HCZ and 0.25µg/ml of other drugs). Calibration plots were constructed by plotting peak areas against the corresponding amount of the drugs.

Method validation

Specificity: The specificity of the method was ascertained by analyzing drugs from standard and sample. The bands for all four drugs from the sample were confirmed by comparing the Rf and UV spectra of the respective band with those that obtained from standard. The peak purity was assessed by comparing UV spectra acquired at three different positions on the band, i.e. peak start (s), peak apex (m) and peak end (e).

Linearity: For TEL, IRB and RAM response was a linear function of amount in the range 250-1250ng per spot, for HCZ the linear range was 500-2500ng per spot. The correlation coefficients, r were 0.9991 for TEL, 0.9991 for HCZ, 0.9992 for RAM and 0.9990 for IRB. The average linear regression equations were calculated and shown in (Table 1 & Figure 1).

Table 1: Linearity Data for all four drugs.

| Drug       | Linearity Range (ng/spot) | R²    | Slope     | Intercept |
|------------|---------------------------|-------|-----------|-----------|
| Telmisartan| 250-1250                  | 0.9991| 394.11    | 25.279    |
| Hydrochlorothiazide | 500-2500             | 0.9991| 1200.7    | 93.564    |
| Ramipril   | 250-1250                  | 0.9992| 510.85    | 51.568    |
| Irbesartan | 250-1250                  | 0.999 | 800.14    | 96.021    |

Precision: Precision was determined by analysis of standard solutions containing concentrations of TEL, HCZ, RAM and IRB covering the entire calibration range. The precision of the method, as intra-day variation (RSD, %) was determined by analysis of these solutions three times on the same day. Inter-day precision (RSD, %) was assessed by analysis of these solutions on three different days over a period of one week. The results of precision studies are shown in (Table 2 & Figure 2).

Table 2: Recovery Data of all four drugs in tablet formulation.

| Drug | Recovery Level (%) | Initial Amount (ng) | Amount Added (ng) | Mean Recovery (%) (n=3) | RSD (%) |
|------|--------------------|---------------------|-------------------|-------------------------|---------|
| TEL  | 80                 | 500                 | 400               | 100.33                  | 0.521   |
|      | 100                | 500                 | 500               | 99.98                   | 0.432   |
|      | 120                | 500                 | 600               | 100.25                  | 0.332   |
| HCZ  | 80                 | 1000                | 800               | 101.31                  | 0.411   |
|      | 100                | 1000                | 1000              | 98.85                   | 0.572   |
|      | 120                | 1000                | 1200              | 100.34                  | 0.38    |
| RAM  | 80                 | 500                 | 400               | 99.85                   | 0.109   |
|      | 100                | 500                 | 500               | 100.08                  | 0.403   |
|      | 120                | 500                 | 600               | 100.21                  | 0.233   |
| IRB  | 80                 | 500                 | 400               | 99.35                   | 0.117   |
|      | 100                | 500                 | 500               | 99.28                   | 0.342   |
|      | 120                | 500                 | 600               | 100.17                  | 0.61    |

Citation: Sivasubramanian L, Lakshmi KS (2017) Simultaneous Estimation of Irbesartan, Telmisartan, Hydrochlorothiazide and Ramipril in Combined Dosage forms by Validated HPTLC Method. J Anal Pharm Res 4(4): 00112. DOI: 10.15406/japlr.2017.04.00112
Simultaneous Estimation of Irbesartan, Telmisartan, Hydrochlorothiazide and Ramipril in Combined Dosage forms by Validated HPTLC Method

Three dimensional densitogram showing linearity and resolution of drugs.

**Accuracy:** The accuracy of the method was determined by analysis of standard additions at three different levels, i.e. multiple-level recovery studies. Sample stock solution obtained from the tablet formulations was spiked with amounts equivalent to 80, 100 and 120% of amounts of drugs in original solution. When these solutions were analyzed the recoveries were found to be within the acceptable limits.

**Sensitivity:** The sensitivity of measurement of TEL, HCZ, IRB and RAM was estimated in terms of limit of quantitation (LOQ). The smallest amounts detected under the chromatographic conditions used were estimated in terms of the limit of detection (LOD). LOD and LOD were calculated by use of the equations LOD = 3.3×N/B and LOQ = 10×N/B, where N is the standard deviation of the peak areas of the drugs, taken as the measure of noise and B is the slope of the corresponding calibration plot.

**Analysis of marketed formulation**

Twenty tablets of each preparation were weighed and powdered. Suitable amount of powder was transferred in to a 50ml volumetric flask. After addition of 3ml of methanol, the solution was centrifuged at 600rpm for 15 min to ensure complete extraction of drugs from matrix. The volume was made up with methanol and filtered through a 0.45µ filter (Millipore, Milford, MA, USA). Standard addition technique was followed for drugs of lesser amount in formulations to meet the calibration limits.

**Results and Discussion**

**Selection of wavelength**

To investigate the appropriate wavelength for simultaneous determination of HCZ, IRB, TEL and RAM, solutions of these compounds in methanol were scanned by UV-visible spectrophotometry (Perkin Elmer Lambda 25 model) in the range of 200-350nm. Suitable wavelength choices were selected for monitoring the drugs in HPTLC system from the overlapped UV spectra. Suitable wavelength choices obtained from the overlapped UV spectra were 260, 230 and 210nm. Solutions of each substance were spotted on the TLC plate and the responses (peak area) were recorded at 260, 230 and 210nm after development. It was observed that 210nm as the most appropriate wavelength for analysis of all the substances with suitable sensitivity.

**Mobile phase optimization**

A variety of mobile phases, for example chloroform-methanol, chloroform-toluene, chloroform-toluene-methanol, chloroform-toluene-acetic acid, acetonitrile-toluene-acetic acid and methanol-toluene-acetic acid mixtures were investigated for separation of TEL, IRB, HCZ and RAM. Finally a mixture of acetonitrile, toluene and methanol gave good resolution, but peak shapes were missing. Formic acid was added to get good peak shape. Addition of small amount of formic acid reduced peak tailing and improved peak characteristics. Acetonitrile toluene: methanol:formic acid 8:10:2:0.6 (v/v/v/v) was found to result in best peak shape. The drugs RAM, TEL, HCZ and IRB were satisfactorily resolved with R, values 0.35, 0.42, 0.54 and 0.60 respectively. Pre-saturation of TLC chamber with mobile phase vapour for 20 min ensured more reproducible migration of the drugs and better resolution.

**Method validation**

**Linearity and range:** Calibration plots were constructed by plotting peak areas against the corresponding amount of the drugs. For TEL, IRB and RAM response was a linear function of amount in the range 250-1250ng per spot, for HCZ the linear range was 500-2500ng per spot. The correlation coefficients, r were 0.9991 for TEL, 0.9991 for HCZ, 0.9992 for RAM and 0.9990 for IRB. The average linear regression equations were calculated and shown in Table.

**Specificity:** Peak purity for the drugs were tested by acquiring spectra at the peak start (S), peak apex (A) and peak end (E) positions. Results from correlation of the spectra were: for RAM, r(S, A) = 0.9996 and r(A,E) = 0.9994, for TEL, r(S, A) = 0.9998 and r(A,E) = 0.9995, for HCZ, r(S, A) = 0.9998 and r(A,E) = 0.9997 and for IRB, r(S, A) = 0.9999 and r(A,E) = 0.9998 respectively. It can thus be concluded that no impurities or degradation products were eluted along with the peaks obtained from the standard drug solution.

**Precision:** The repeatability of sample application was assessed by application of 1µl of standard drug solution seven times to an HPTLC plate, development of plate, and recording peak height and peak area of the zones. The RSD (%) of peak height and area were 0.65 and 0.54 respectively for RAM, 0.55 and 0.43 for HCZ, 0.33 and 0.43 for TEL and 0.21 and 0.42 for IRB.

Repeatability of measurement of peak height and area were determined by application of 1µl standard drug solution to an HPTLC plate, developing the plate, and recording peak height and area of the zones. The RSD (%) of peak height and area were 0.21 and 0.13 respectively for RAM, 0.08 and 0.11 for HCZ, 0.04 and 0.12 for TEL and 0.09 and 0.05 for IRB.

**Accuracy:** The accuracy of the method was determined by analysis of standard additions at three different levels, i.e. multiple-level recovery studies. Sample stock solution obtained from the tablet formulations was spiked with amounts equivalent to 80, 100 and 120% of amounts of drugs in original solution. When these solutions were analyzed the recoveries were found to be within the acceptable limits ranging from 98.85% to 101.31% for all four drugs.

**Sensitivity:** LOQ and LOD were calculated by use of the equations LOD =3.3×N/B and LOQ = 10×N/B, where N is the standard deviation of the peak areas.
deviation of the peak areas of the drugs, taken as the measure of noise and B is the slope of the corresponding calibration plot. LOQ and LOD for TEL were 136.23 and 44.38ng, for RAM they were 168.33 and 56.78ng, for IRB they were 180.54 and 65.66ng, for HCZ they were 268.44 and 98.55ng respectively.

**Assay of marketed formulation**

The applicability of the method was verified by the determination of all the drugs in respective formulations. The content of all the drugs were found to be within the acceptance limit and are mentioned in (Table 3 & 4) (Figure 3). No interference from the excipients present in the marketed formulations was observed. The low % RSD value indicated the suitability of the method for routine analysis of all four drugs in various formulations.

| Drug | Concentration (ng per Spot) | Intra-Day Precision (RSD, %, n=3) | Inter-Day Precision (RSD, %, n=3) |
|------|-----------------------------|-----------------------------------|-----------------------------------|
| TEL  | 250                         | 0.823                             | 1.231                             |
|      | 500                         | 0.674                             | 0.984                             |
|      | 750                         | 0.543                             | 1.134                             |
| HCZ  | 500                         | 0.456                             | 0.873                             |
|      | 1000                        | 0.762                             | 0.985                             |
|      | 1500                        | 0.342                             | 0.885                             |
| RAM  | 250                         | 0.566                             | 1.203                             |
|      | 500                         | 0.672                             | 1.073                             |
|      | 750                         | 0.487                             | 0.983                             |
| IRB  | 250                         | 0.771                             | 1.332                             |
|      | 500                         | 0.652                             | 1.07                              |
|      | 750                         | 0.472                             | 0.768                             |

**Table 4: Analysis of Marketed Formulation.**

| Drug     | Label Claim (mg/Tablet) | Amount Founda (mg) | Drug Contenta (%) | Standard Deviation (SD) | RSD (%) | SE |
|----------|-------------------------|--------------------|-------------------|-------------------------|---------|----|
| XARB H (Formulation 1)                |                      |                    |                   |                         |         |    |
| IRB     | 150                     | 150.43             | 100.28            | 0.402                   | 0.4     | 0.131 |
| HCZ     | 12.5                    | 12.58              | 100.64            | 0.543                   | 0.539   | 0.21  |
| Telista H (Formulation 2)             |                      |                    |                   |                         |         |    |
| TEL     | 40                      | 40.56              | 101.4             | 0.344                   | 0.339   | 0.231 |
| HCZ     | 12.5                    | 12.57              | 100.56            | 0.441                   | 0.438   | 0.208 |
| Telma R5 (Formulation 3)              |                      |                    |                   |                         |         |    |
| TEL     | 40                      | 40.14              | 100.35            | 0.624                   | 0.621   | 0.312 |
| RAM     | 5                       | 4.96               | 99.2              | 0.562                   | 0.566   | 0.22  |
| Cardace # 2.5 (Formulation 4)         |                      |                    |                   |                         |         |    |
| RAM     | 2.5                     | 2.53               | 101.2             | 0.076                   | 0.075   | 0.125 |
| HCZ     | 12.5                    | 12.46              | 99.68             | 0.354                   | 0.355   | 0.233 |

* Average of six estimations:

T1: XARB H-Nicholas Piramal, Mumbai.
Irbesartan: 150mg and Hydrochlorothiazide-12.5mg.
T2: Telista H-Lupin Pharma, Mumbai.
Telmisartan: 40mg and Hydrochlorothiazide-12.5mg.
T3: Telma R5-Glenmark, Mumbai.
Telmisartan: 40mg and Ramipril-5mg.
T3: Cardace # 2.5-Aventis Pharma, Goa.
Ramipril: 2.5mg and Hydrochlorothiazide-12.5mg.

**Citation:** Sivasubramanian L, Lakshmi KS (2017) Simultaneous Estimation of Irbesartan, Telmisartan, Hydrochlorothiazide and Ramipril in Combined Dosage forms by Validated HPTLC Method. J Anal Pharm Res 4(4): 00112. DOI: 10.15406/japlr.2017.04.00112
Simultaneous Estimation of Irbesartan, Telmisartan, Hydrochlorothiazide and Ramipril in Combined Dosage forms by Validated HPTLC Method

Conclusion

HPTLC method was developed for estimation of IRB, HCZ, TEL and RAM in tablet formulation. The method was validated as per ICH (Q2 R1) guidelines. The proposed method was found to be specific, accurate, precise and sensitive. The developed method was successfully applied for quantitative analysis of all four drugs in their combined tablet formulation. Results were found to be in good agreement with label claim of their combined tablet formulation. The proposed method can be applied for routine analysis of IRB, HCZ, TEL and RAM in their combined dosage form.

Acknowledgements

The authors are thankful to the management of SRM University for providing the facilities to carry out the work. The authors are also thankful to Madras Pharmaceuticals, Chennai for providing the standard drugs as gift samples.

References

1. Lakshmi S, Lakshmi KS (2014) H-Point Standard Addition Method for Simultaneous Spectrophotometric Determination of Irbesartan, hydrochlorothiazide and Telmisartan in tablets. IPRC 4(2): 373-380.
2. Srinivasa RK, Minakshi P, Nargesh KK (2011) Spectrophotometric methods for the simultaneous estimation of losartan potassium and hydrochlorothiazide in tablet dosage forms. Chronicles of young scientists 2(3): 155-160.
3. Sunil S, Ajit KY, Hemendra G (2011) Simultaneous Estimation of valsartan and hydrochlorothiazide solid dosage form using UV Spectroscopy. Bulletin of Pharmaceutical Research 1(3): 10-12.
4. Rahman N, Ahmad Y, Azmi SNH (2005) Kinetic Spectrophotometric Determination of Ramipril in Pharmaceutical Formulations. AAPS Pharm Sci Tech 6(3): E543-551.

Figure 3: Densitogram of Marketed Formulations.

XARB H: Irbesartan (150 mg) and Hydrochlorothiazide (12.5 mg).
Telista H: Telmisartan (40 mg) and Hydrochlorothiazide (12.5 mg).
Telma R: Telmisartan (40 mg) and Ramipril (2.5 mg).
Cardace #2.5: Ramipril (2.5 mg) and Hydrochlorothiazide (12.5 mg).
Simultaneous Estimation of Irbesartan, Telmisartan, Hydrochlorothiazide and Ramipril in Combined Dosage forms by Validated HPTLC Method

5. Chaitali T, Jyoti D, Pawr PY (2014) Simultaneous estimation and validation of losartan potassium and hydrochlorothiazide in bulk and tablet dosage form using different spectrophotometric method. Der Pharma Chemica 6(2):24-30.

6. El-Gindy A, Ashour A, Abdel-Fattah L, Shabana MM (2001) Spectrophotometric determination of benazepril hydrochloride and hydrochlorothiazide in binary mixture using second derivative, second derivative of the ratio spectra and chemometric methods. J Pharm Biomed Anal 25(2):299-307.

7. Attia MS (2001) Spectrofluorimetric Assessment of Ramipril using Optical Sensor Samarium Ion-Dipyridyl Complex Doped in Sol-Gel Matrix. J Pharm Biomed Anal 51(1): 7-11.

8. Abdellatef HE (2007) Spectrophotometric and Spectrofluorimetric Methods for the Determination of Ramipril in its Pure and Dosage Form, Spectrochim. Acta A Mol Biomol Spectrosc 66(3):701–706.

9. Nagub IA, Abdelakem EA, Draz ME, Zaazaa HE (2014) Linear Support Vector regression and partial least squares chemometric models for determination of hydrochlorothiazide and Bencapril hydrochloride in presence of related impurities: a comparative study. Spectrochim Acta A Mol Biomol Spectrosc 113: 350-356.

10. Darwish HW, Hassan SA, Salem MY, El- Zeany BA (2013) Comparative study between derivative spectrophotometry and multivariate calibration as analytical tools applied for the simultaneous quantitation of amlodipine, valsartan and Hydrochlorothiazide. Spectrochim Acta A Mol Biomol Spectrosc 113: 350-356.

11. Lakshmi KS, Lakshmi Sivasubramanian (2011) Simultaneous spectrophotometric determination of valsartan and hydrochlorothiazide by H-point standard addition method and partial least square regression. Acta Pharm 61(1):37-50.

12. Hegazy MA, Metwaly FH, Abdelkawy M, Abdelwahab NS (2010) Spectrophotometric and chemometric determination of hydrochlorothiazide and spironolactone in binary mixture in the presence of their impurities and degradants. Drug Test Anal 2(5): 243-251.

13. Kaya Beliz, Erdal Dinc, Dumitru Baleanu (2009) Chemometric methods for the simultaneous spectrophotometric determination of telmisartan and hydrochlorothiazide in the commercial pharmaceuticals. Rev Chem 60(6): 544-550.

14. Dinc E, Ustundag O (2003) Spectrophotometric quantitative resolution of hydrochlorothiazide and spironolactone in tablets by chemometric analysis methods. Farmaco 59(11): 1151-1161.

15. Alazazi AM, Abdelhameed AS, Khalil AA, Dharwish IA (2014) HPLC method with monolithic column for simultaneous determination of irbesartan and hydrochlorothiazide in tablets. Acta Pharm 64(2): 187-198.

16. Koyturturk S, Can NO, Atkosar Z, Arli G (2014) A novel dilute and shoot HPLC assay method for quantification of irbesartan and hydrochlorothiazide in combination tablets and urine using second generation C18 bonded monolithic silica column with double gradient elution. J Pharm Biomed Anal 97: 103-110.

17. Goswami N (2014) A validated stability indicating liquid chromatographic method for determination of process related impurities and degradation behaviour of irbesartan in solid dosage form. J Adv Pharm Tech Res 5(1): 33-40.

18. Vujic Z, Mulavidi N, Smajic M, Brboric J, Stankovic P (2012) Simultaneous analysis of irbesartan and hydrochlorothiazide an improved HPLC method with the aid of the chromometric protocol. Molecules 17(3): 3461-3474.

19. Rawool ND, Venkatachalam A (2011) Analytical method for the simultaneous estimation of hydrochlorothiazide and metropolitartate using RP-HPLC. Indian J Pharm Sci 73(2): 219-223.

20. Anand Babu K, Vinoth Kumar G, Lakshmi Sivasubramanian (2011) Simultaneous estimation of Ramipril and Amlopidine in pharmaceutical dosage form by RP-HPLC method. Int J Pharm Pharm Sci 3(4): 196-198.

21. Bae SK, Kim MJ, Shim EJ, Cho DY, Shon JH, et al. (2009) HPLC Determination of Irbesartan in Human Plasma: Its Application to Pharmacokinetic Studies. Biomed Chromatogr 23: 569-572.

22. Ferreirós N, Iriarte G, Alonso RM, Jiménez E, Ortiz E (2008) Separation and Quantification of Several Angiotensin II Receptor Antagonist Drugs in Human Urine by a SPE-HPLC-DAD Method. J Sep Sci 31(4): 667-676.

23. Najma S, Saeed AM, Shahid AS, Shahnawaz S (2008) Simultaneous Determination of Olmesartan Medoxomil and Irbesartan and Hydrochlorothiazide in Pharmaceutical Formulations and Human Serum using High Performance Liquid Chromatography. Chin J Chromatogr 26: 544-549.

24. Ivanovic D, Medenica M, Jančetti B, Knezevic N, Malenovic A, et al. (2007) Validation of analytical procedure for simultaneous determination of hydrochlorothiazide and lisinopril and their impurities. Acta chromatographica 18: 143-156.

25. Ferreirós N, Iriarte G, Alonso RM, Jiménez RM (2007) Development of a Solid Phase Extraction Procedure for HPLC-DAD Determination of Several Angiotensin II Receptor Antagonists in Human Urine using Mixture Design. Talanta 73(4): 748-756.

26. Shalaya AK, Al-Hiari YM, Alhamamib OMO (2007) Liquid Chromatographic Determination of Irbesartan in Human Plasma. J Chromatogr B 848: 245-250.

27. Patel LJ, Suhagia RN, Shah PB, Shah RR (2006) Simultaneous estimation of bisoprolol fumarate and hydrochlorothiazide in tablet dosage form by RP-HPLC method. Indian J Pharm Sci 85(5): 635-638.

28. Caudron E, Laurent S, BILLAUD EM, Prognon P (2004) Simultaneous Determination of the Acid/Base Antihypertensive Drugs Captopril, Bisoprolol and Irbesartan in Human Plasma by Liquid Chromatography. J Chromatogr B 801(2): 339-345.

29. Erk N (2003) Simultaneous Determination of Irbesartan and Hydrochlorothiazide in Human Plasma by Liquid Chromatography. J Chromatogr B 784(1): 195-201.

30. Nie J, Zhang M, Fan Y, Wen Y, Xiang B, et al. (2003) Biocompatible In-Tube Solid Phase Microextraction Coupled to HPLC for the Determination of Angiotensin II Receptor Antagonists in Human Plasma and Urine. J Chromatogr B 828(1-2): 62-69.

31. González L, López JA, Alonso RM, Jiménez RM (2002) Fast Screening Method for the Determination of Angiotensin II Receptor Antagonists in Human Plasma by High-Performance Liquid Chromatography with Fluorimetric Detection. J Chromatogr A 949(1-2): 49-60.

32. Hertzog DL, McCafferty JF, Fang X, Tyrrell RJ, Reed RA (2002) Development and validation of a stability-indicating HPLC method for the simultaneous determination of Losartan potassium, hydrochlorothiazide, and their degradation products. J Pharm Biomed Anal 30(3): 747-760.

33. Erk N (2001) Analysis of binary mixtures of losartan potassium and hydrochlorothiazide by using high performance liquid chromatography, ratio derivative spectrophotometric and compensation technique. J Pharm Biomed Anal 24(4): 603-611.
Simultaneous Estimation of Irbesartan, Telmisartan, Hydrochlorothiazide and Ramipril in Combined Dosage forms by Validated HPTLC Method

34. Belal F, Al-Zaagi IA, Gadkariem EA, Abounaassif MA (2001) A Stability Indicating LC Method for the Determination of Ramipril and Hydrochlorothiazide in Dosage Forms. J Pharm Biomed Anal 24(3): 335-342.

35. Hogan BL, Williams M, Idicula A, Veysooglu T, Parente E (2000) Development and Validation of a Liquid Chromatographic Method for the Determination of the Related Substances of Ramipril in Atlee Capsules. J Pharm Biomed Anal 23(4): 637-651.

36. Qui X, Wang Z, Wang B, Zhan H, Pan X, et al. (2014) Simultaneous determination of irbesartan and hydrochlorothiazide in human plasma by ultra high performance liquid chromatography tandem mass spectrometry and its application to the bioequivalence study J Chromatogr B Anal Technol Biomed Life Sci 957: 110-115.

37. Chi-Yu Lu, Chia-Hsien Feng (2011) Quantitation of Irbesartan and Major Proteins in Human Plasma by Mass Spectrometry with Time-of-Flight Analyzer. J Pharm Biomed Anal 54(1): 100-105.

38. Prasaja B, Sasongko L, Harahap Y, Hardiyanti, Lusthom W, et al. (2009) Simultaneous quantification of Losartan and active metabolite in human plasma by LC-MS using irbesartan as internal standard. J Pharm Biomed Anal 49(3): 862-867.

39. Lee HW, Ji HY, Park ES, Lee KC, Lee HS (2009) Hydrophilic Interaction Chromatography–Tandem Mass Spectrometric Analysis of Irbesartan in Human Plasma: Application to Pharmacokinetic Study of Irbesartan. J Sep Sci 32(14): 2353-2358.

40. Sathe SR, Bari SB (2007) Simultaneous analysis of losartan potassium, atenolol, and hydrochlorothiazide in bulk and in tablets by high-performance thin-layer chromatography with UV absorption densitometry. Acta Chromatographica 19: 270-278.

41. Kristoffersen L, Øiestad E, Opdal MS, Krogh M, Lundanes E, et al. (2007) Simultaneous Determination of 6 Beta-Blockers, 3 Calcium Channel Antagonists, 4 Angiotensin-II Antagonists and 1 Antiarrhythmic Drug in Post-Mortem Whole Blood by Automated Solid Phase Extraction and Liquid Chromatography Mass Spectrometry. Method Development and Robustness Testing by Experimental Design. J Chromatogr B Analys Technol Biomed Life Sci 850(1-2): 147-160.

42. Zhang M, Wei F, Zhang YF, Nie J, Feng YQ (2006) Novel Polymer Monolith Micro extraction using a Poly (Methacrylic Acid-Ethylene Glycol Dimethacrylate) Monolith and its Application to Simultaneous Analysis of Several Angiotensin- II Receptor Antagonists in Human Urine by Capillary Zone Electrophoresis. J Chromatogr A 1102(1-2): 294-301.

43. Sharma M, Kothari C, Sherikar O, Mehta P (2014) Concurrent estimation of amlodipine besylate, hydrochlorothiazide and valsartan by RP-HPLC, HPTLC and UV Spectrophotometry. J Chromatogr Sci 52(1): 27-35.

44. Lakshmi KS, Lakshmi Sivasubramanian (2012) Simultaneous Analysis of Losartan Potassium, Amlodipine Besylate and Hydrochlorothiazide in bulk and in tablets by HPTLC with UV Absorption Densitometry. Journal of Analytical Methods in Chemistry 2012: 1-5.

45. Kumbar ST, Chougule GK, Tegeli VS, Gegeli GB (2011) A validated HPTLC method for simultaneous quantification of nebivolol and hydrochlorothiazide in bulk and tablet formulation. Int J Pharm Sci and drug Res 3(1): 62-66.

46. Panchal HJ, Suhagia BN (2010) Simultaneous determination of Atorvastatin calcium and ramipril in capsule dosage forms by HPLC and HPTLC. J AOAC Int 93(5): 1450-1457.

47. Lakshmi KS, Lakshmi Sivasubramanian, Krishanu Pal (2010) Stability indicating HPTLC method for simultaneous determination of Ramipril and Telmisartan in tablets. Int J Pharm Pharm Sci 2(4): 127-129.

Citation: Sivasubramanian L, Lakshmi KS (2017) Simultaneous Estimation of Irbesartan, Telmisartan, Hydrochlorothiazide and Ramipril in Combined Dosage forms by Validated HPTLC Method. J Anal Pharm Res 4(4): 00112. DOI: 10.15406/japlr.2017.04.00112