CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187

https://zenodo.org/records/10397335

Available online at: http://www.iajps.com *Research Article*

# STABILITY INDICATING HPTLC METHOD FOR DETERMINATION TIMOLOL MALEATE IN BULK DRUG & ITS FORMULATION

**Adawale Sanjana Sanjay*, Dr.Dhumal . P. B, Dr. Sameer Shafi, Dr. Vishweshwar Dharashive.**

Shivlingeshwar College of Pharmacy, Almala, Taluka. Ausa, Dist. Latur

**Abstract:**

*Timolol was the first β blocker to be used as an anti-glaucoma agent and to date remains as the standard because none of the newer β blockers were found to be more effective. A new, simple, precise, accurate and sensitive High Performance Thin Layer Chromatographic method has been developed for the estimation of Timolol Maleate in bulk and marketed ophthalmic formulation. Chloroform: Methanol: Ammonia (9:1:0.1 v/v/v) was selected as a mobile phase. The determination was made at 296 nm for Timolol Maleate over the concentration range of 100-1000 ng/spot. The Correlation Coefficient $R^2$ in calibration curve was found to be 0.9949. The $R_f$ value was found to be 0.53 ± 0.027. The intra-day and inter-day relative standard deviations are in the range 0.55-0.99 % and 0.47-1.85 % respectively. The validation of method was carried out as per ICH Guidelines. The stability-indicating ability and specificity of the proposed method forced degradation studies were performed. Hydrolysis under acidic, alkaline & neutral & oxidative & thermal and photolytic degradation studies were conducted on Timolol Maleate. No interference of additives, matrix etc. is encountered in these methods. The drug showed 10- 20 % degradation in acidic, alkali, neutral, oxidation, thermal & Photolytic conditions. stability indicating Statistical analysis proved that these methods are reproducible, selective for the analysis of Timolol Maleate in bulk drug and in its ophthalmic formulations and can be used to determine the purity of the drug even in presence of excipients and related impurities.*

***Keywords:*** *Timolol Maleate, High Performance Thin Layer Chromatographic, Validation, Degradation.*

**Corresponding author:**

**Miss. Adawale Sanjana Sanjay,**

*Shivlingeshwar College of Pharmacy, Almala, Taluka. Ausa, Dist. Latur*

***Email - sanjanaadawale881@gmail.com***

*Contact No – 8830003885*

QR code

*Please cite this article in press Adawale Sanjana Sanjay et al.,* ***Stability Indicating HPTLC Method For Determination Timolol Maleate In Bulk Drug & Its Formulation ,*** *Indo Am. J. P. Sci, 2023; 10 (12).*

## INTRODUCTION:

The analysis of drug substance (API) and drug product (Formulation made by adding drug substance to appropriate excipients) is simply termed as pharmaceutical analysis. The aim of pharmaceutical analysis is to acquire data by analysing bulk drug materials, the intermediates of their syntheses, products of drug research (potential pharmacons), drug formulations, the impurities and degradation products of drugs, biological samples containing the drugs and their metabolites. The data can contribute in the achievement of high quality, maximum efficacy and safety of drug therapy and maximum economy of the production of drugs

[1] The assessment of safety, efficacy, and quality of the pharmaceutical product to match the regulatory standards requires well-planned studies and testing with suitable methodologies and instrumentation. The assurance of the quality of the finished product is not the sole criteria that is to be met for regulatory requirements. The quality assessment at various stages of the development process is mandatory for successful outcome. Modern pharmaceutical analysis is not only limited to the assessment of the finished pharmaceutical product but it also plays a crucial role in each stage of the drug discovery process. The demand for analytical data under sample-limited conditions and in full compliance of current Good Manufacturing Practices (cGMP) has made the analytical department a significant partner in the drug development process.

[2] The work of a pharmaceutical analyst begins with the evaluation of structure of new chemical entity (NCE) or new molecular entity (NME). Verification of the purity of product from contaminants like by-products, interaction products or degradation products is also essential since impurities may exert side effects.The assessment of toxicological and safety behaviour.
chemicals used were of Analytical Reagent (AR) grade.

## MATERIALS AND METHODS:

Timolol Maleate was kindly gifted by Astron Research, Centre, Ahmedabad and BrimonidineTartarte was kindly gifted by FDC Ltd.., Mumbai. All other reagents and chemicals used were of Analytical Reagent (AR) grade.

A new, simple, precise, accurate and sensitive High Performance Thin Layer Chromatographic method has been developed for the estimation of Timolol Maleate in bulk and marketed ophthalmic formulation. Chloroform: Methanol: Ammonia (9:1:0.1 v/v/v) was selected as a mobile phase.

The drug used for present investigation was obtained from Astitva Chemicls as gift sample.

- **HPTLC Instrumentation**

Camag Linomat V (Semiautomatic Spotting device), Camag Twin trough Chamber (10 × 10 cm2), Camag TLC Scanner-3, Camag win CATS v.1.3.4 Software, Hamilton Syringe (100 µl), Shimandzu Libror AEG – 220 balance. The samples were spotted in the form of bands of width 6 mm with a Camag 100 µl sample (Hamilton, Bonaduz., Switzerland) syringe on TLC aluminium sheet pre-coated with silica gel G 60 F254 (10 cm x 10 cm with 0.2 mm thickness) using a Camag Linomat applicator 5 (Switzerland). A constant application rate of 10 µl sec-1 was employed and space between two bands was 10 mm. The slit dimension was kept 4 mm x 0.45 mm micro. The mobile phase consisted of hexane: iso-propyl alcohol: ammonia (5.0:2.0:0.5 v/v). The optimized chamber saturation time for mobile phase was 20 min at room temp (25°C±2) and relative humidity 60%±5. The length of chromatogram run was approximately 70 mm. Subsequent to the development; TLC plates were dried in current of air with the help of an air dryer. Densitometric scanning was performed using Camag TLC scanner 3 in the absorbance mode at 281 nm. The source of radiation utilized was mercury lamp emitting a continuous UV spectrum in the range of 200-400 nm.

HPTLC Instrumentation
CamagLinomat V (Semiautomatic Spotting device), CamagTwin trough Chamber (10 × 10 cm2), Camag TLC Scanner-3, Camag winCATS v.1.3.4 Software, Hamilton Syringe (100 µl), ShimandzuLibror AEG – 220 balance.
The samples were spotted in the form of bands of width 6 mm with a Camag 100 µl sample (Hamilton, Bonaduz., Switzerland) syringe on TLC aluminium sheet pre-coated with silica gel G 60

PTLC Instrumentation
Camag Linomat V (Semiautomatic Spotting device), CamagTwin trough Chamber (10 × 10 cm2), Camag TLC Scanner-3, Camag win CATS v.1.3.4 Software, Hamilton Syringe (100 µl), Shimandzu Libror AEG – 220 balances.

The samples were spotted in the form of bands of width 6 mm with a Camag 100 µl sample (Hamilton, Bonaduz., Switzerland) syringe on TLC aluminium sheet pre-coated
with silica gel

**Methods**

**•Melting point**

Melting point of drug was determined by placing small amount of sample in capillary tube closed at one end and holds the capillary on melting point apparatus. The melting point was noted and readings were depicted in Table No.07.

- **FT-IR analysis:**

Infrared (IR) spectroscopy is an absorption method widely used in both qualitative and quantitative analyses. The infrared region of the spectrum includes electromagnetic radiation that can alter the vibrational and rotational states of covalent bonds in organic molecules. The IR absorbance spectrum of Timolol Maleate was recorded using FTIR 8400S spectrometer (Shimadzu) over range of 4000 to 400 cm-1

**1. Preparation of standard stock solution:**

Timolol Maleate (10 mg) was weighed accurately and transferred to 10 ml volumetric flask. It was dissolved in 10 ml of methanol to obtain concentration of 1000 µg/ml. 0.1 ml of the above solution was further diluted with methanol to obtain the concentration 10 µg/ml of Timolol Maleate.

**2. Preparation of sample stock solution:**

For preparing sample stock solution, 1ml of eye drop were taken (each ml contain 5 mg of Timolol Maleate). The 1ml eye drop dissolved in 5 ml of methanol to obtain the concentration of 1000 µg/ml. The solution was sonicated for 15 min. The solution wasfiltered through Whatman filter paper no.41, and volume was adjusted to 10 ml with the same solvent. 0.1 ml of this solution was further diluted with methanol to obtain the concentration 10 µg/ml of Timolol Maleate.

**3. Chromatographic separation:**

Four micro-litre of standard solution of Timolol Maleate (10 µg/ml) was applied on prewashed and activated plate under nitrogen stream using semiautomatic spotter. It was developed at constant temperature in a Camag twin-trough chamber previously saturated for 20 min with mobile phase

- **Validation of method**

The method was validated as per the ICH guidelines in terms of linearity, accuracy and specificity, limit of detection, limit of quantitation, intra-day and inter-day precision, repeatability of measurement of peak area as well as repeatability of sample application. Limit of detection and limit of quantitation: Decreasing amount of drug substance was applied in triplicate on the plate, developed and scanned as described earlier. The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value. b) Selection of analytical concentration range: (calibration, linearity &range) A stock solution of Timolol Maleate (10ng/µl) was prepared in methanol.

**1. Precision**:

**Intra-day precision**: The intra-day precision was determined by analysing standard solutions of Timolol Maleate three different concentrations in the concentration range of 40-120 ng/spot for three times on the same day. Each concentration was applied in triplicate and % RSD was calculated.

Accuracy: (Recovery studies) Recovery studies was carried out by applying the method to drug sample to which known amount of Timolol Maleate corresponding to 80, 100 and 120 % of label claim had been added (standard addition method). At each level of the amount, six determinations were performed ad the results obtained were compared with expected results.

**2. Robustness of the method:**

The robustness of the method was studied, during method development at three different concentration levels of 50 ng/spot, 150 ng/spot and 200 ng/spot, by determining the effects of small variation of mobile phase composition (±0.1 %), amount of mobile phase (±5 %), time from spotting to chromatography (±20 min) and scanning time (±20 min). The % RSD of peak area for each parameter was calculated.

**3. Forced Degradation**

The stability-indicating method is the quantitative analytical methods that are based on the characteristic structural, chemical or biological properties of each ingredient of a product and that will distinguish each active ingredient from its degradation product so that the active ingredient content can be accurately measured.

**4. Preparation of Acid and Base Induced – Degradation Product**

To 10 mL of stock solution, 10 mL each of 1N HCl and 0.01N NaOH were added separately. These mixtures were refluxed for 2 hr and 2.5 hr at 70 ºC respectively. The forced degradation in acidic and basic media was performed in the dark inorder to exclude the g/mLµpossible degradative effect of light. The resultant solution was diluted to obtain 10 L were injected into the system and the chromatograms were recorded toµsolution and 20 assess the stability of sample.

## RESULTS AND DISCUSSION:

**1. Melting point range determination**

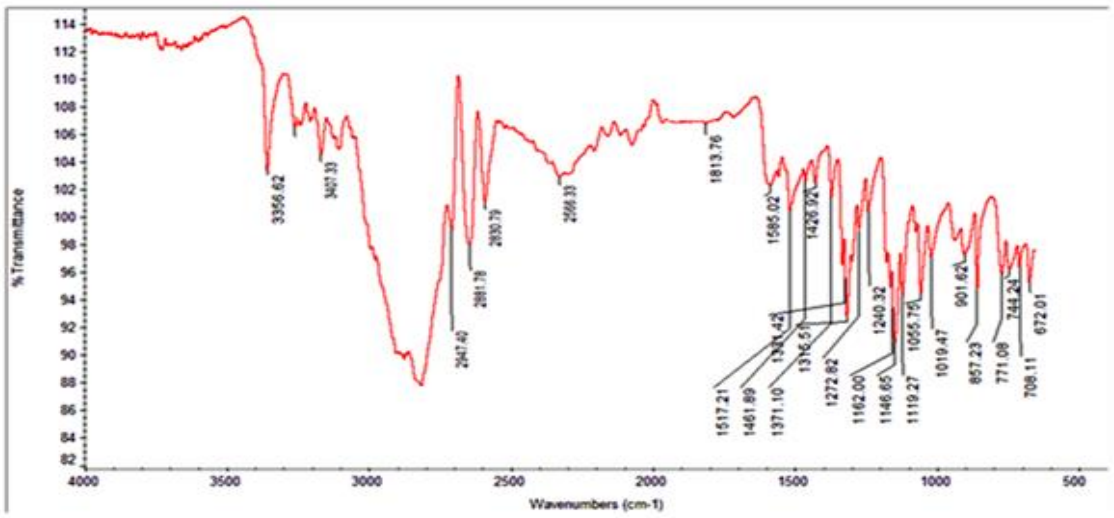
Melting point of drug was determined by placing small amount of sample in capillary tube closed at one end and holds the capillary on melting point apparatus. The melting point was noted and readings were depicted in table.

**Table: Melting point range analysis result**

| Sr. No. | Name of Drug | Melting point |
|---|---|---|
| 1 | Timolol Maleate | 202-203°C |

**2. FT-IR analysis:**

The IR absorbance spectrum of Timolol Maleate was recorded using FTIR 8400S spectrometer (Shimadzu) over range of 4000 to 400 cm-1.




**3. UV spectroscopy analysis:**

The ultraviolet absorption spectrum of Timolol Maleate was obtained using Shimadzu1800- UV visible spectrophotometer and 1cm quartz cells, over a wavelength range of 400 to 200 nm. The wavelength maxima (λmax) were analyzed showed in table no.06

**Table: Drug wavelength maxima (λmax)**

| Sr. No. | Name of Drug | Observed value (λ max ) nm |
|---|---|---|
| 1 | Timolol Maleate | 296 |

**4. Chromatographic Condition:**

- Stationary phase: Precoated silica gel G60 F254 aluminium sheets 10X10 cm2, layer thickness 0.2mm.
- Activation: TLC plates prewashed with Methanol and activated in oven at 60°C for 10 mins.
- Mobile phase: Chloroform: Methanol: Ammonia (9:1:0.1 v/v/v)
- Temperature: Room temperature TLC chamber saturation
- Time: 15 min Migration distance: 6 cm
- Detection: Densitometrically using a UV detector at 296nm
- praying rate: 10 µl/sec
- Scanning parameters:
- Slit dimension: 5 x 0.20 mm
- Wavelength of detection: 296 nm
- Lamp: Deuterium
- $R_f$ value 0.53 ± 0.027

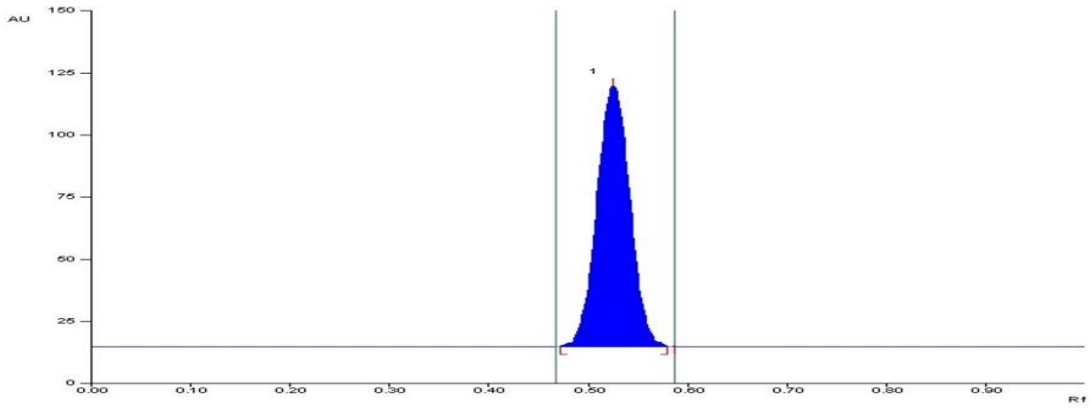



**Figure : Dentiform of standard Timolol Maleate; ($R_f$, 0.53 ± 0.027)**

**Assay of marketed formulation:**

The sample for formulation i.e. eye drop Timoletwere taken for analysis. The % drug content of Timolol Maleate was found to be 101.15±0.68. the results shown in following table no 12

**Table No 12: Assay of Timolol Maleate**

| Drug | Label Claim (mg) | % of Label Claim | SD* | % RSD* |
|---|---|---|---|---|
| Timolol Maleate | 5 | 101.15 | 4.547 | 0.044 |

- **Validation**

**1. Precision:**

Precision of an analytical method is expressed as S.D or R.S.D of series of measurements. It was ascertained by replicate estimation of the drugs by proposed method.

**Intra-day precision:**

**Table: Intra-day& Inter Day precision of Timolol Maleate**

| Precision | % of Label Claim | SD | % RSD |
|---|---|---|---|
| Intraday (n=3×3 ) | 101.935 | 5.360 | 0.052 |
| Interday (n= 3×3) | 102.789 | 4.871 | 0.047 |

**Table: Repeatability of sample application of Timolol Maleate**

| Concentration (ng) | Area | | Average Area | SD | % RSD* |
|---|---|---|---|---|---|
| | Plate 1 | Plate 2 | | | |
| 100 | 633.29 | 649.56 | 641.42 | 5.1 | 0.35 |
| 100 | 628.61 | 657.49 | 643.05 | | |
| 100 | 619.54 | 639.15 | 629.34 | | |
| 100 | 635.34 | 640.37 | 637.86 | | |
| 100 | 639.52 | 642.19 | 640.86 | | |
| 100 | 610.64 | 659.10 | 634.87 | | |

**2. Specificity**

To confirm the specificity of the proposed method, Timolol Maleate was spotted on TLC plate, It was observed that excipients present in formulation did not interfere with peak of Timolol Maleate ($R_f$, 0.53±0.01).

**3. Recovery**

The % mean recovery was found to be 100.64±0.11 for Timolol Maleatewhich indicate that the proposed method is accurate for estimation of drug in formulation.

**Table: Recovery of Timolol Maleate**

| Drug | Amount taken (ng per band) | Amount added (ng/band) | Total amount (ng/band) | % Recovery* | % RSD* |
|---|---|---|---|---|---|
| TIMO | 200 | 100 | 300 | 100.193 | 1.851 |
| | 200 | 200 | 400 | 101.406 | 0.647 |
| | 200 | 300 | 500 | 100.082 | 0.944 |

**4. Robustness**

The robustness of the method was studied, by changing various parameters during method development. The % RSD of peak area for each parameter was calculated. The results are depicted in table no 16.

**Table: Robustness of Timolol Maleate**

| Sr.No. | Parameters | Variation | % RSD* |
|---|---|---|---|
| 1. | Chamber saturation period | ± 10 % | 1.09 |
| 2. | Time from application to development | 0, 10, 20, 30 min | 0.069 |
| 3. | Time from development to scanning | 0, 30, 60, 90 min | 0.055 |

- **Stability in sample solution**

To provide an indication of the stability-indicating ability and specificity of the proposed method forced degradation studies were performed. Hydrolysis under acidic, alkaline & neutral & oxidative & thermal and photolytic degradation studies were conducted on Timolol Maleate

1. **Acid degradation**

For acid decomposition studies, 1 ml of pure drug solution (concentration 1 mg/ml) was mixed with 1 ml of 1 M MethanolicHCl and volume was made up to 10 ml with methanol and solution was refluxed for 12 hr. 10 µl volume was applied on TLC plate to obtain the chromatogram. Under this condition 2 peaks of degradation products were observed.

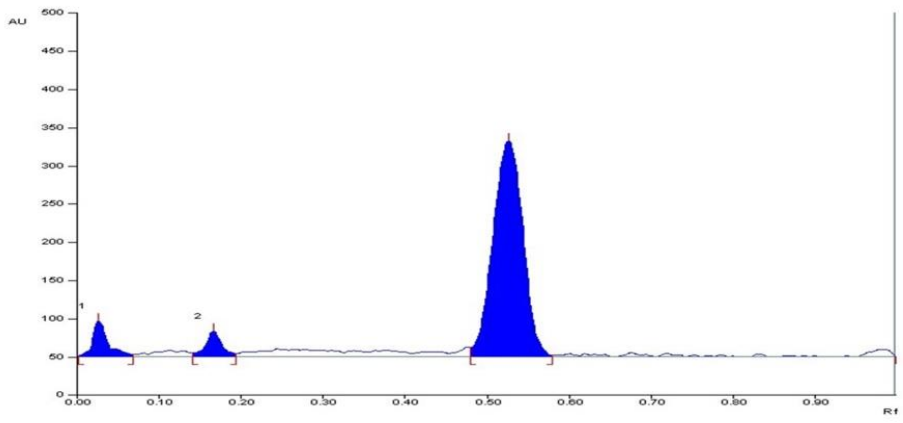



**Figure: Chromatogram showing the separation of different degradation products of TIMOobtained under acidic condition**

**2. Base Degradation**

1 ml of pure drug solution (concentration 1 mg/ml) was mixed with 1 ml of 1 M Methanolic NaOH and volume was made up to 10 ml with water and solution was refluxed 12 hr. 10 µl volume was applied on TLC plate to obtain the chromatogram. Under this condition 3 peak of degradation product was observed.

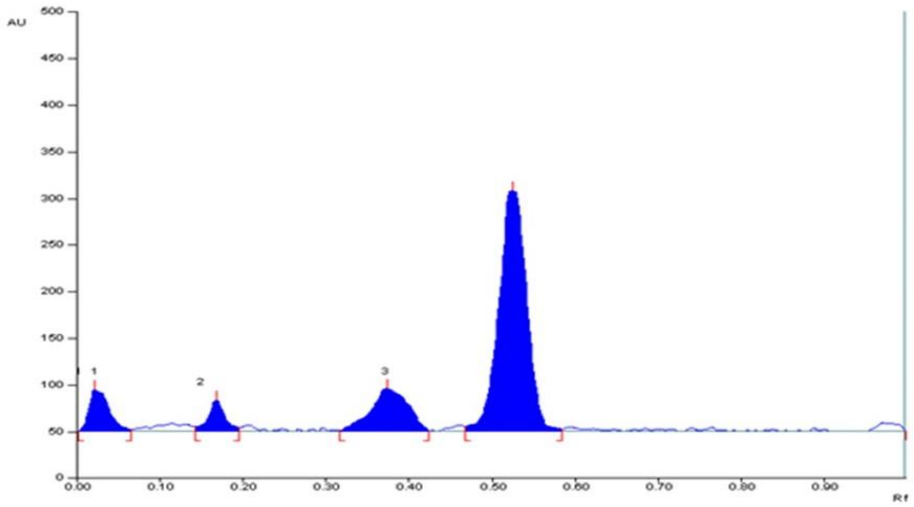



**Figure: Chromatogram showing the separation of different degradation products of TIMO obtained under alkaline condition**

## CONCLUSION:

From the studies it can be concluded that HPTLC technique can be successfully used for the estimation of the Timolol Maleate and in its formulations. The method shows good reproducibility, the HPTLC method is accurate, precise, specific, reproducible, and sensitive.

No interference of additives, matrix etc. is encountered in these methods. The drug showed 10-20 % degradation in acidic, alkali, neutral, oxidation, thermal & Photolytic conditions. stability indicating Statistical analysis proved that these methods are reproducible, selective for the analysis of Timolol Maleate in bulk drug and in its ophthalmic formulations and can be used to determine the purity of the drug even in presence of excipients and related impurities. The HPTLC method proved to be simple, less expensive, fast, accurate, precise and robust and thus can be used for routine analysis of Timolol Maleate in ophthalmic solutions. Further studies on other pharmaceutical formulations would throw more light on these studies.

This study can be extended to study the degradation kinetics of Timolol Maleate and for estimation in plasma and other biological fluids.

## REFERENCES:

1) ICH, Q2 (R1), Validation of Analytical Procedures: Text and Methodology, ICHHarmonizedTripartite Guidelines, Geneva,(2005).
2) FDA, Guidance for Industry: Bio analytical Method Validation. US Departmentof Health and Human Services, Food and Drug Administration, Centre for DrugEvaluation and Research (CDER), and Centre for Veterinary Medicine (CVM),(2001).
3) http://www.who.int/governance/eb/who_constitution_en.pdf (Accessed on 6thApril2017)
4) S.Gorog,J.Pharm. Biomed.Anal.,48 (2008)247–253.
5) S Ahuja and S Scypinski, Handbook of Modern Pharmaceutical Analysis,SeparationScienceandTechnology,AcademicPress,USA(2011).
6) M. R. Siddiqui, Z. A. AlOthman, N. Rahman, Arab. J. Chem., 10 (2017) S1409–S1421.
7) J. Schappler, V. Gonzalez-Ruiz, S. Rudaz, CE-MS in Drug Analysis andBioanalysis, In G. de Jong ed. Capillary Electrophoresis - Mass Spectrometry(CE-MS): Principles and Applications, John Wiley & Sons, New Jersey, (2016),p. 129-158
8) R.W.LeeandL.Goldman,TechnicalBrief,Particlesciences,PA,USA,vol.5(2009)1-2.
9) D.Guillarme,J.Schappler,J.Boccard,J.L.Veuthey, S.Rudaz,Chimia.66(2012)330-334.
10) GorogS,J. Pharm. Biomed. Anal. 36(2005)931-937.
11) JErmer, J.Pharm. Biomed. Anal.24 (2001)755–767.

12) I. Bak, Modern analytical techniques in the pharmaceutical and bioanalysis,University of Debrecen Medical and Health Science Center Faculty ofPharmacy Department of Pharmacology Pharmacutical and bioanalyticaldivision,Hungary,(2011).
13) A.M. Sameer, K. AbdulrahmanBasavaiah, 2011. Chem. Ind. Chem. Eng. Q. 17(2011)173–178.
14) N. Matei, S. Birghila, V. Popescu, S. Dobrinas, A. Soceanu, C. Oprea, V.Magearu,Rom. J. Phys.53 (2008)343–351.
15) E. Rutkowska, K. Pajak and K. Jozwiak, Acta. Pol. Pharm. Drug. Res., 70(2013)3-18.