Novel HPTLC and UV-AUC analyses: For simple, economical, and rapid determination of Zileuton racemate

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Zileuton; HPTLC; Sodium lauryl sulphate; Hydrotropy; UV-Spectroscopic-Area under curve

Abstract
Novel, simple, rapid and reliable High-Performance Thin-Layer Chromatographic (HPTLC) and UV-spectroscopic area under curve (UV-AUC) methods were developed and validated for the analysis of zileuton racemate in bulk and in-house tablet formulation. HPTLC quantitation of zileuton was done by UV detection at 260 nm and analysis was performed on (20 × 10 cm) aluminium sheets precoated with silica gel 60-F 254 (E. Merck) as stationary phase and toluene–methanol–glacial acetic acid (3.5:1.5:0.1 v/v) as mobile phase. Quantitation by HPTLC method resulted into a compact and well resolved band for zileuton at retention factor \( R_f \) of 0.51 ± 0.02. Linear regression analysis data for calibration of HPTLC method represented a good linear relationship with regression coefficient; \( r^2 = 0.997 \). UV-AUC method was developed using sodium lauryl sulphate (0.05 M) as a hydrotropic agent to enhance water solubility and area was determined at a wavelength range in between 248.40 and 271.0 nm. Correlation coefficient for UV-AUC analysis was found to be \( r^2 = 0.999 \). The developed UV-AUC method depicted a fine linear relationship for zileuton racemate in a concentration range of 2–12 \( \mu \)g/mL. Both the developed methods were validated for precision, robustness, ruggedness, accuracy, sensitivity as per guidelines laid by the International Conference on Harmonisation (ICH). Statistical analysis proved that the developed methods were precise, robust, sensitive and accurate and can be used effectively for the analysis of zileuton in bulk and pharmaceutical formulations.

1. Introduction

Inflammatory diseases akin to psoriasis, rheumatoid arthritis, asthma, multiple sclerosis, uveitis and inflammatory bowel syndrome are implicated by leukotrienes produced via arachidonic acid metabolism by 5-lypoxygenase. These leukotrienes (LT’s) are, unstable LTA4; followed by its sequential metabolism to LTB4, LTC4, LTD4 and LTE4 (Rask-Madson et al., 1992) which bind to the specific receptors in airways (Lewis
Zileuton \( R, S(\pm) \) N-(1-(benzo[\( b\) ]-thien-2-yl)ethyl)-N-hydroxyurea (Fig. 1) is a racemic mixture having approximately equal therapeutic activities (Erdman, 1992). Zileuton selectively and reversibly inhibits 5-lipoxygenase activity and forms a complex with enzyme’s iron molecule in the active site and inhibits calcium-ionophore-stimulated human neutrophil production of LTB4 after oral dosing (McGill and Busse, 1996). Zileuton is a very slightly soluble compound without any ionisable functional group (Qui et al., 1997) and approved in the United States to treat asthma. A recent report in the literature has emphasised review of zileuton as a new efficient and safe anti-acne drug (Zouboulis, 2009). Optimum absorption for zileuton has been reported and majority of the dose is recovered in the urine of healthy volunteers as the N-hydroxyl glucuronides of both the \( R(+) \) and \( S(-) \) enantiomers (Wong et al., 1995). Zileuton is metabolised in the presence of NADPH (Awni et al., 1995a) and it is a weak inhibitor of CYP1A2 (Lu et al., 2003). The drug has several clinically important drug interactions such as inhibiting warfarin and theophylline metabolism (Awni et al., 1995b; Granneman et al., 1995a). Zileuton also showed a significant reduction in; infiltration of inflammatory cells and incidence of hyperplasia (Yang et al., 2008).

Zileuton is official in the United States pharmacopeia (USP) and its assay is proposed there in via HPLC with application of ammonium acetate-acetohydroxamic acid buffer solution adjusted to pH 2.0 with perchloric acid and acetonitrile as a mobile phase in a ratio of (72:28, v/v) (United States Pharmacopeia 34-NF 29, 2011).

Literature survey revealed very critical, varied and atypical reports for efficient analytical determinations for zileuton viz… Preparative separation and analysis of the enantiomers of \([^{14}C]\) zileuton (Thomas and Surber, 1992a), Kinetics and mechanism of chemical degradation in aqueous solutions by HPLC (Thomas and Surber, 1992b), HPLC determination with its N-dehydroxylated metabolite in plasma (Granneman et al., 1995b), simultaneous determination along with N-dehydroxylated metabolite in untreated rat urine using HPLC (Thomas and Albazi, 1996), electrochemical reduction behaviour of zileuton at a dropping mercury electrode by polarography (Shreedhar et al., 2010) and one report on solubility and stability characterisation of zileuton in a ternary solvent system assisted with HPLC (Trivedi et al., 1996). The published data depict no literature for the analysis of zileuton by High-Performance Thin-Layer Chromatography (HPTLC) and simple UV-spectroscopy. The pharmaceopial method of analysis by HPLC is supposed to use solvents in large quantities along with higher concentrations of buffers in high proportion; requiring greater time for the analytical determination and may lead to some restrictions in analysis. On the other hand developed HPTLC method will require lesser quantities of solvents allowing cost-effective analysis with a provision to analyse large number of samples in a short period. Zileuton racemate is practically water insoluble hence; we thought to use a hydrotropic agent for the complete dissolution of drug and to perform its UV-AUC analysis. A peak at 260 nm was small; lacking sharpness and uniformity; so, to avoid any possible lacunae in spectroscopic analysis and to increase the reproducibility and accuracy of the developed UV-AUC method we measured the uniform areas under the curves in between wavelengths 248.40 and 271.0 nm. This might have assisted us to detect the drug and develop its spectrophotometric method at a low concentration range of 2–12 \( \mu \)g/mL again.

Hence, proposed investigation was intended to put forth the establishment of a novel; simple, efficient, rapid and economical analysis of zileuton with application of HPTLC and UV-AUC analyses to assist a routine quality control of zileuton in bulk and pharmaceutical formulations.

2. Experimental

2.1. Drugs and reagents

Zileuton standard was obtained as a gift sample from Biophore India Ltd. (Hyderabad, India), Methanol (HPLC Grade), Sodium Lauryl Sulphate (SLS), Sodium Starch Glycolate (SSG), and Microcrystalline Cellulose (MCC) used during the study were procured from (Loba Chemie, India). Water used for chromatography and spectroscopy was double-distilled water and used throughout the experimental work described. Tablets containing 600 mg of zileuton were prepared in-house.

2.2. Equipments and experimental conditions

2.2.1. For HPTLC analysis

A Camag TLC system (Muttenz, Switzerland) comprising of Camag Linomat 5 automatic sample applicator, Hamilton syringe (100 \( \mu \)L), Camag TLC scanner 3, Camag winCATS software (version 1.3.0), Camag twin trough chamber (20 \times 10 cm) and ultrasonicator; ENERTECH Electronics Pvt. Ltd., India was used during the study.

Chromatographic study was carried out on aluminium-backed precoated silica gel 60-F \( 254 \) (20 \times 10 cm) HPTLC plates having 200 \( \mu \)m thickness (E. Merck, Mumbai, India). Prior to use; the HPTLC plates were pre-washed and dried in oven at 100 °C. Densitometric detection was performed with a Camag TLC Scanner 3 (Camag, Muttenz, Switzerland) installed with winCATS software. Drug standards and samples were applied on the HPTLC plates using Linomat 5 (Camag) applicator under nitrogen gas flow. Samples (10 \( \mu \)L) were spotted 6 mm from the edge of the plates. The plates were developed in a twin trough glass chamber (20 \times 10 cm) (Camag, Muttenz, Switzerland). The volume of the mobile phase was 10 mL. Mobile phase components were mixed prior to use and the development chamber was left for saturation with mobile phase vapours for 20 min before each run at room temperature (25 °C ± 2). Development of the plate was carried out by the ascending technique to a migration distance of 8 cm followed by drying with the help of an air drier. The slit dimensions were kept at 6.00 \( \times \) 0.45 mm (micro) and scanning speed employed was 20 mm s\(^{-1}\). Densitometric scanning was done in absorbance-reflectance mode at 260 nm using a deuterium lamp emitting a continuous UV-spectrum between 190 and 400 nm. The slit dimensions were set at 6 mm.

![Figure 1](image-url) 

**Figure 1** Chemical structure of zileuton.

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2.2.2. For UV-AUC analysis

A UV–Visible spectrophotometer (2450 Shimadzu, software UV Probe 2.21) with a spectral bandwidth 1 nm was employed for all spectroscopic measurements, using a pair of 10 mm matched quartz cells. 0.05 M SLS was employed as solvent for UV spectrophotometric determination of zileuton which in particular has the advantage that it is also an official dissolution media for zileuton tablet formulation mentioned in USP to predict the in vitro release characteristics of zileuton (United States Pharmacopeia 34-NF 29, 2011).

SLS (0.05 M) was used as a hydrotropic agent and wetting agent to enhance the solubility of poorly water soluble zileuton racemate. Surfactant property of SLS assisted us to make the drug completely soluble in double distilled water. SLS is well known for its surfactant, wetting, spreading action and used to assist dissolution of poorly water soluble drugs as well as to predict in vitro release profiles of sparingly water soluble drugs (Zonggui et al., 2004).

2.3. Preparation of standards

2.3.1. For HPTLC analysis

Zileuton 20 mg was weighed with assistance of SHIMADZU AUX-120 analytical balance and dissolved in a 100 mL volumetric flask containing 25 mL of methanol; sonicated for 10 min using ultrasonicator ENERTECH Electronics Pvt. Ltd., India and the volume was made up to the mark to get a stock standard solution of 0.2 mg/mL of zileuton. From the standard solution, appropriate volumes of 1–6 mL were transferred with the help of previously calibrated pipette (10.0 mL) into a series of 10 mL volumetric flasks. A fixed volume of 10 μL was applied on the HPTLC plates to obtain concentrations 200, 400, 600, 800, 1000 and 1200 ng per band of zileuton, respectively. Each concentration was applied six times to the plates and plates were developed as described before in Section 2.2.1. Peak areas were plotted against their corresponding concentrations to obtain the calibration plot.

2.3.2. For UV-AUC analysis

Zileuton is practically insoluble in water, 0.05 M SLS was employed as a solvent for complete dissolution of drug in water and development of spectral characteristics of drug. SLS is used as a hydrotropic agent to increase the solubility of very slightly soluble zileuton in water. A stock standard solution of zileuton was prepared by accurately weighing 10 mg of zileuton as described in Section 2.3.1 followed by its dissolution in 25 mL 0.05 M SLS followed by sonication in ultrasonicator for 15 min to remove the frothing produced if any and the volume was made up to the mark very slowly by adding 0.05 M SLS to obtain a final concentration of 100 μg/mL for standard solution used during UV-AUC analysis. Care was taken and dilutions were made with very slow addition of 0.05 M SLS to prevent the formation of foam and to avoid possible flaws in the analysis that may occur due to foam and foam formation.
entrapped air. After appropriate dilutions, 10 µg/mL zileuton was scanned in the UV-region i.e., 400–200 nm.

2.4. Preparation of samples

Due to the unavailability of zileuton tablets in the local Indian market, In-house tablets were formulated via direct compression technique using SSG and MCC containing 600 mg of drug per tablet.

To determine the content of in-house prepared tablets of Zileuton, twenty tablets were weighed as described before in Section 2.3.1 and powdered in a glass mortar. An amount of powder equivalent to 50 mg zileuton was transferred into 100 mL volumetric flask containing 25 mL of methanol, sonicated for 20 min, and solution was diluted to volume with same solvent. The resulting solution was filtered through a 0.45 µm filter (Millifilter, Milford, MA, USA).

For HPTLC determinations 8 µL from filtrate was applied on HPTLC plates to perform tablet assay for the drug.

For UV-spectroscopic determination an amount of powder equivalent to 50 mg zileuton was transferred into 100 mL volumetric flask containing 50 mL of 0.05 M SLS, sonicated for 20 min and solution was diluted to volume with same solvent and same precautions. The resulting solution was filtered through a 0.45 µm filter slowly and a volume of filtrate equal to 160 µL of solution was transferred into 10 mL volumetric flask and the volume was made up to mark with 0.05 M SLS to obtain the final concentration of 8 µg/mL zileuton. The resulting solution was subjected to the proposed method and the amount of zileuton present was determined.

3. Results and discussion

The optimised HPTLC and UV-Spectroscopic-AUC methods were validated as per standard procedures to ensure them for linearity, precision, selectivity, sensitivity, robustness, accuracy and specificity.

3.1. Development of optimum mobile phase

In order to obtain high resolution and reproducible peaks different mobile phase compositions were tested. The required parameters were found optimum with use of toluene-methanol-glacial acetic acid (3.5:1.5:0.1 v/v) as mobile phase. The wavelength of 260 nm was found to be optimal for the highest sensitivity. A sharp and well resolved peak was obtained for zileuton at \( R_f \) of 0.51 ± 0.02 when the chamber was saturated with mobile phase for 20 min at room temperature.

3.2. Linearity and calibration curve

Linearity study during HPTLC involved; use of 200 µg/mL of zileuton as a stock standard and constant volume of 10 µL were spotted on HPTLC plates to obtain 200, 400, 600, 800, 1000 and 1200 ng/band of zileuton, respectively. The calibration curve of area versus concentration was found to be linear in the range of 200–1200 ng/band. Zileuton depicted a good linear relationship of area against concentrations evaluated during HPTLC analysis. Linear regression equation for

\[
y = 0.176 x + 0.005 \\
\left( r^2 = 0.999 \right)
\]

Figure 3 Zero order spectrum of zileuton standard depicting AUC in between 248.40 and 271.0 nm in 0.05 M SLS.

Figure 4 Overlaid spectra of linearity study during UV-AUC analysis of zileuton in 0.05 M SLS.
HPTLC was found to be $y = 2.432x + 1560$ with regression coefficient ($r^2 = 0.997$) which is generally considered as evidence of acceptable fit. The results are shown in Fig. 2.

UV-AUC method was validated for linearity by preparing working standards from stock standard solution containing 100 µg/mL of zileuton to obtain solutions containing 2–12 µg/mL of zileuton and scanned in UV region 400–200 nm against blank solution of 0.05 M SLS. Zileuton showed maximum absorption ($\lambda_{\text{max}}$) at 260 nm. Wavelengths 248.40 and 271 nm were selected for the measurement of area under curve (AUC) Fig. 3. The calibration curve of AUC versus concentration was found to be linear in the range of 2–12 µg/mL. Linear regression equation for UV-AUC was found to be $y=0.176x+0.005$ with regression coefficient ($r^2=0.999$). Results are as quoted along with overlain spectra Fig. 4.

3.3. Precision

Repeatability and intermediate precision are two essential components for the determination of precision during analytical method validation which indicates the ease of analysis for the operator in a laboratory while striving to obtain same results for the same batch of material using same developed method at a different time with application of same equipments and reagents.

The intra-day and inter-day precisions of the HPTLC and UV-AUC analyses were evaluated using linear regression data for the calibration curve; analysing zileuton repeatedly at concentrations of 400, 800 and 1000 ng/band for HPTLC analysis and 6, 8 and 10 µg/mL for UV-AUC determination. The precision of the developed HPTLC and UV-AUC methods is expressed in terms of percent relative standard deviation (% RSD). The % RSD value was found to be within the range. Results depicted optimum precision for both analyses and data are represented as in Table 1.

3.4. Limits of detection and quantification

The limit of detection (LOD) and limit of quantification (LOQ) determinations for both specified methods were based on the standard deviations of the responses and slopes of constructed calibration curves ($n = 3$) as described by the International Conference for Harmonisation guidelines Q2(R1) (ICH, 1997, addendum incorporated 2005).

For the determination of LOD and LOQ during HPTLC method validation; zileuton solutions of 200, 240, 280, 320, 360 and 400 ng/band were applied on HPTLC plates. Determination of LOD and LOQ for UV-AUC analysis involved use of concentrations towards lower end; 2.0, 2.5, 3.0, 3.5 and 4.0 µg/mL from the linearity range. The LOD and LOQ were calculated using equations $\text{LOD} = 3.3 \times \text{N/B}$ and $\text{LOQ} = 10 \times \text{N/B}$; Where, ‘N’ is standard deviation of peak areas of the drug ($n = 3$) taken as a measure of noise and ‘B’ is the slope of corresponding calibration curve. As a result minimum concentrations at which analyte can be reliably detected and quantified were calculated and found to be 24 and 86 ng for HPTLC and 0.45 and 1.46 µg for UV-AUC analysis, respectively.

3.5. Robustness

Robustness of an analytical method is a measure of its tendency to confront deliberate variations in method parameters. The robustness was demonstrated by performing test method in normal condition and each altered condition mentioned below.

### Table 1 Precision studies for zileuton racemate for HPTLC and UV-AUC analyses.

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Drug Conc. (ng/band)</th>
<th>Amount found (ng/band)</th>
<th>% RSD</th>
<th>Amount found (ng/band)</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPTLC</td>
<td>400</td>
<td>399.84</td>
<td>0.99</td>
<td>386.4</td>
<td>1.01</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>792.6</td>
<td>0.53</td>
<td>789.4</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>986.8</td>
<td>0.46</td>
<td>984.2</td>
<td>0.89</td>
</tr>
<tr>
<td>UV-AUC</td>
<td>6</td>
<td>5.99</td>
<td>1.21</td>
<td>6.12</td>
<td>1.83</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>8.12</td>
<td>1.72</td>
<td>8.14</td>
<td>1.39</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>9.84</td>
<td>1.36</td>
<td>10.06</td>
<td>1.84</td>
</tr>
</tbody>
</table>

* Mean of three estimations at each level.

### Table 2 Robustness for HPTLC analysis of zileuton racemate [$n = 3$].

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Peak area ± SD</th>
<th>RSD [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase composition (±0.3 mL)</td>
<td>21.88</td>
<td>1.24</td>
</tr>
<tr>
<td>Mobile phase volume (±2 mL)</td>
<td>24.27</td>
<td>1.80</td>
</tr>
<tr>
<td>Development distance (±0.5 cm)</td>
<td>30.02</td>
<td>1.83</td>
</tr>
<tr>
<td>Plate saturation time (±5 min)</td>
<td>31.11</td>
<td>1.06</td>
</tr>
<tr>
<td>Relative humidity (±5%)</td>
<td>22.23</td>
<td>1.76</td>
</tr>
<tr>
<td>Activation of HPTLC plates previously developed with methanol and dried at 60°C (±2 min)</td>
<td>19.99</td>
<td>1.24</td>
</tr>
<tr>
<td>Time from application to chromatography (±10 min)</td>
<td>28.49</td>
<td>1.77</td>
</tr>
<tr>
<td>Time from chromatography to scanning (±10 min)</td>
<td>28.86</td>
<td>1.68</td>
</tr>
</tbody>
</table>

$n$ – Number of estimations.
The composition of the mobile phase was changed slightly and the effects on the results were examined. Toluene: methanol: glacial acetic acid in different ratios (3.2:1.8:0.2, 3.8:1.2:0.2, v/v) were selected (keeping volume of glacial acetic acid same) and chromatograms were run. The amount of mobile phase (10 ± 2 mL, i.e. 8, and 12 mL), development distance (8 ± 0.5 cm, i.e. 7.5 and 8.5 cm) and duration of saturation (20 ± 5 min, i.e. 15, 20 and 25) were varied. Time from application of zileuton to the plate to development of the plate and time from development of plate to scanning were also varied (10, 20, or 30 min) and results are depicted in Table 2.

Table 3 Recovery studies for zileuton racemate for HPTLC and UV-AUC analyses.

<table>
<thead>
<tr>
<th>In-house</th>
<th>Initial amount of drug (600 mg/tab)</th>
<th>Amount of standard drug added (%)</th>
<th>% Recovery [n = 3]</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPTLC</td>
<td>600 (ng/band)</td>
<td>80</td>
<td>99.89</td>
<td>1.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>100.4</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>100.2</td>
<td>1.33</td>
</tr>
<tr>
<td>UV-AUC</td>
<td>8 (µg/mL)</td>
<td>80</td>
<td>100.7</td>
<td>1.79</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>99.81</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>99.74</td>
<td>1.37</td>
</tr>
</tbody>
</table>

n – Number of estimations at each level.

Figure 5 HPTLC chromatogram for zileuton standard in toluene-methanol-glacial acetic acid (3.5:1.5:0.1 v/v) as mobile phase.

Figure 6 UV-Spectra for comparison of zileuton standard and zileuton extracted from tablets during HPTLC analysis.
3.6. Accuracy

Accuracy of the methods was evaluated by spiking the drug at three concentration levels 80%, 100% and 120% in a placebo and determining the recovery of the drug added. The data for recovery are represented in Table 3, good recoveries in the range of 99.89% – 100.4% and 99.74% – 100.7% of the drug were found after analysing by developed; HPTLC and UV-AUC methods at various added concentrations, despite the fact that the drug was fortified to a placebo consisting of SSG and MCC.

3.7. Specificity for HPTLC

A typical absorption spectrum of zileuton is shown in Fig. 5. The peak-purity of zileuton was tested by correlating the spectra of zileuton added to placebo and zileuton standard at the peak-start (S), peak-apex (A) and at the peak-end (E) positions. Correlation between these spectra indicated purity of zileuton peak {correlation $r(S, M) = 0.9999$, $r(M, E) = 0.9995$} Fig. 6.

3.8. Ruggedness

Ruggedness of HPTLC method was performed at a concentration of 800 ng/band and for UV-AUC it was determined by using drug at a concentration of 8 µg/mL. Methods were found to be rugged when analysis was performed by two different analysts under the same experimental and environmental conditions.

3.9. Assay of in-house prepared zileuton tablets

A single peak at $R_f$ of 0.51 ± 0.02 was observed in the chromatogram for zileuton during HPTLC analysis. There was no interference observed from the excipients used in the formulation of in-house zileuton tablets. The drug content ± SD found for HPTLC analysis was 98.96 ± 1.84 and for UV-AUC analysis it was found to be 99.84 ± 1.08.

The summary of regression, validation and in-house tablet assay parameters is represented in Table 4 for developed HPTLC and UV-AUC analyses.

### Table 4 Summary of Regression, validation and in-house tablet formulation assay parameters for HPTLC and UV-AUC analyses.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>RP-HPTLC</th>
<th>UV-AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression coefficient</td>
<td>0.997</td>
<td>0.999</td>
</tr>
<tr>
<td>Slope ± SD</td>
<td>2.432 ± 0.102</td>
<td>0.176 ± 0.006</td>
</tr>
<tr>
<td>Intercept ± SD</td>
<td>1500 ± 36.02</td>
<td>0.005 ± 0.001</td>
</tr>
<tr>
<td>Concentration range</td>
<td>200–1200 [ng/band]</td>
<td>2–12 [µg/ml]</td>
</tr>
<tr>
<td>Intra-day precision (n = 3, RSD,%)</td>
<td>0.66</td>
<td>1.43</td>
</tr>
<tr>
<td>Inter-day precision (n = 3, RSD,%)</td>
<td>1.23</td>
<td>1.68</td>
</tr>
<tr>
<td>Accuracy (n = 9, RSD,%)</td>
<td>100.16%; 1.37</td>
<td>99.87%; 1.32</td>
</tr>
<tr>
<td>LOD</td>
<td>24 ng</td>
<td>0.45 µg</td>
</tr>
<tr>
<td>LOQ</td>
<td>86 ng</td>
<td>1.46 µg</td>
</tr>
<tr>
<td>Robustness</td>
<td>Robust</td>
<td>Robust</td>
</tr>
<tr>
<td>Drug content</td>
<td>98.96 ± 1.84</td>
<td>99.84 ± 1.08</td>
</tr>
</tbody>
</table>

$n$ – Number of estimations.

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### References

