Development of ledipasvir and sofosbuvir pure certified reference materials for improving quality of pharmaceutical analysis

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Abstract: The pure materials play a pivotal role in controlling the quality of pharmaceutical products, ensuring comparability and metrological traceability of test results, and performance evaluation of measuring instruments. Herein, the purity characterization of chronic hepatitis C virus sofosbuvir and ledipasvir pharmaceuticals was established based on the assessment of mass fractions of potential impurities in the material including heavy metals, residual solvents, and water content, as well as structurally related organic impurities. The combined estimate of these impurities by mass balance approach led to indirect purity determination of sofosbuvir and ledipasvir materials. The impurities mass fractions in each material were assigned by validated chromatographic methods, Karl Fisher titrator, and inductively coupled plasma-based method. The certified purity values of sofosbuvir and ledipasvir and their corresponding expanded uncertainties (k =2.0 at 95% CL) were found (99.79±0.03) %, (99.69±0.04) %, respectively. The developed certified reference materials (CRMs) with small uncertainty values will support pharmaceutical testing laboratories in their efforts to maintain and improve the quality of results and provide them with high-order CRMs for the accurate determination of both analytes in raw materials and finished products.

Keywords: Ledipasvir, sofosbuvir, value assignment, mass balance approach, certified reference materials. © 2022 ACG Publications. All rights reserved.

1. Introduction

In 2014, US Food and Drug Administration (FDA) and European Medicines Agency has approved of the fixed dose combination of the NS5B polymerase inhibitor sofosbuvir and the NS5A inhibitor ledipasvir for the treatment of chronic hepatitis C virus (HCV) genotype 1a and 1b infection [1-6]. HCV infection is a global health issue with approximately 185 million people affected worldwide, and infection in Egypt was reported as the highest prevalence in the world [7-9]. This approval leads to an increase in production of ledipasvir–sofosbuvir based medicines, as well as demand of certified reference materials (CRMs) for quantitative analysis. Well certified and characterized pure reference materials are needed for controlling the quality of pharmaceutical products, calibration and performance check of measuring instruments, internal quality control, interlaboratory comparisons, and monitoring the competence of laboratory personnel [10-19]. Additionally, the application of certified reference materials (CRMs) in testing laboratories is required to establish metrological traceability of measurement results to SI units in order to operate in conformity with the requirements of ISO/IEC 17025:2017 [19-22]. The traceability of
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pharmaceutical measurement results to the SI is generally achieved through linkage to pure reference materials. The purity of the materials is defined by the amount of desired analyte in a material relative to the total amount of all analytes, a material is sufficiently pure when amount of each of the impurities which may interfere with the specific purpose for which the material is required is so low and their combined effect is negligible within the desired limits of accuracy [23-25]. Presently, there is a lack of availability of ISO17034 well characterized and certified ledipasvir and sofosbuvir pure materials suitable for use as primary reference materials for establishing traceability to SI units [19, 26]. To certify pure reference materials, characterization should be based on metrologically valid approach directly using primary methods or indirectly by characterization and determination of all impurities including water content, volatile organic carbons, residual solvents, structurally-related organic impurities, anions and heavy metals [27-30]. In this context, indirect characterization of ledipasvir and sofosbuvir by metrologically valid approach in accordance to the requirements of ISO 17034:2016 [26] and ISO Guide 35:2017 [30] has been conducted including assessment of homogeneity, stability, characterizations, value assignment and uncertainty estimation. The certification of the materials was based on measurement of water content, volatile organic carbons, residual solvents, structurally-related organic impurities, anions and heavy metals by Karl Fisher titrator (KF), HS-GC-FID, HS-GC-MS, high performance liquid chromatography coupled to diode array detector (HPLC-DAD), liquid chromatography- high resolution mass spectrometer (LC-MS/MS), ion chromatography and inductively coupled plasma- optical emission spectrometer (ICP-OES).

2. Experimental

2.1. Reagent and Chemicals

HPLC grade solvents, methanol, Ethanol, n-propanol, iso-propanol, acetonitrile and acetone were purchased from Sigma-Aldrich (Germany). Pure water with conductivity of 0.055 µS/cm and 5 ppb TOC was obtained through a Milli-Q water purification system (Millipore, Bedford, MA, USA). Nitric acid, ammonium acetate, formic acid, sodium carbonate and sodium bicarbonate were purchased from Merck (Germany). ICP multi-element standard solution was obtained from AccuStandard (USA). Anions standard solutions SRM 3181, SRM 3182, SRM 3183, SRM 3184, SRM 3185, SRM 3186 were obtained from NIST (USA) (see Table S1 for details in supporting information). Karl Fisher reagents, Aquagent complete 5 and Hydranal Coulomat AG was obtained from Scharlau (Spain). Water and organic solvents were used in all procedures without further purification.

2.2. Raw Materials

Fifty grams of ledipasvir and sofosbuvir bulk materials were obtained from NATCO pharma limited (India) and Laurrus Labs (India), respectively. The materials were packed in 0.5 g brown glass vials sealed with aluminium crimp caps with PTFE septa and capped tightly to avoid any adsorption of moisture or change of materials composition. Each batch consisted of approximately 100 vials, the vials were labelled according to packing sequence, divided into ten groups, and each group includes 10 units. All vials were stored at 4 °C for further studies.

2.3. Equipment and Measurement Procedures

2.3.1. Identity and Organic Impurities Determination

Agilent 1100 liquid chromatography integrated system equipped with G1313A automated injector, G1311A quaternary pump and G1315B diode-array detector (DAD) operated at a wavelength of 260 and 330 nm was used for sofosbuvir and ledipasvir, respectively. The chromatographic separation of the compounds was achieved with a reversed phase column, ZORBAX XDB-C18 (5.0 x 250, 5 µm)) from Agilent (USA) operating at constant temperature (30 °C). 50 mg samples were dissolved in 10 mL of Eluent A and Eluent B (50:50), filtered through a 0.45 µm nylon filter and injected into the HPLC. The samples were eluted using two eluants consist of water: methanol: acetonitrile: ammonium acetate (Eluent A, 1:15:4: 0.5 g/L; Eluent B, 36:3:1: 0.5 g/L) at a flow rate of 1 mL/minute and linear gradient started by
10% A and 90% B to 90% A and 10% B after 45 min. The materials were reanalysed by Waters LC-MS/MS equipped with ACQUITY UPLC, Xevo TQD triple quadrupole mass spectrometry and BEH C18 Column (1.7 µm - 2.1 x 50 mm). The samples were eluted by 0.1% formic acid in water (A) and acetonitrile (B) at a flow rate of 0.2 mL/min, and elution program started by 90% A decreased to 70% after 3 min. then 30% after 7 min., then 10% after 11 min. and kept constant for 4 min. The chromatographic data was analysed using Agilent chemstation and Waters empower software.

2.3.2. VOCs and Residual Solvents Determination

Gas chromatography Agilent 6890N equipped with a 7683B automated injector, flame ionization detector (FID), 7694E headspace sampler and 5975 inert XL mass selective detector. The chromatographic separation of suspected VOCs and residual solvents was achieved with a nonpolar column, DB-VRX (60 m, ID 0.25 mm and film thickness 0.25 µm) from J&W Scientific (USA). Helium was used as a carrier gas at constant flow 1.0 mL/min. Oven temperature was started at 50 ℃ and held isothermal for 5 min then increased by 5 ℃ /min to 230 ℃ and held isothermal for 5 min. Injector, ion source, quadruple and interface temperatures were 250 ℃, 230 ℃, 150 ℃ and 290 ℃, respectively. Six vials were selected from each batch, the content of each vial (0.5 g) was transferred to headspace vials for measurement without any further treatments.

2.3.3. Anion Determination

Approximately 100 mg sample was extracted using 10 mL of ultra-pure water, the mixture was shaken for two hours, the mixture was then centrifuged at 5000 rpm for 10 min, and the aqueous extract was filtered by 0.45 µm PTFA syringe filter and directly injected into Dionex ICS-1000 Ion chromatograph equipped with Dionex ASRS- ULTRA suppressor, IonPac AS14 columns of 4 x 250 mm.

2.3.4. Heavy Metals Determination

Three vials were selected randomly from each batch and three portions from each vial were analysed. Approximately 0.1 g of sample was accurately weighed into a clean teflon beaker and 25 ml of nitric acid (80% (v/v)) was added with continuous stirring for 10 min., the samples were directly measured in triplicates by inductively coupled plasma optical emission spectrometer (ICP-OES, Agilent, model 5100) without further dilution or treatment.

2.3.5. Water Content Determination

Water content determination was carried out using Metrohm 852 Titrand0 KF system equipped with diaphragmed cell generator and 803 stirrers. Fluka Hydranal Coulomat AG as the titration solution for coulometric measurements. Six vials were randomly selected from each batch, three separate portions from each vial were transferred to KF coulometric titration cell for measurement without any treatments.

2.4. Homogeneity Testing

The homogeneity study was designed in accordance with ISO Guide 35 to determine within and between vials heterogeneity of the prepared material. This guide recommends that 10% of prepared vials should be selected to evaluate homogeneity of small batches. Thus, 10 vials were selected randomly from each of the ledipasvir and sofosbuvir batches, selected vials were analysed by HPLC. Three sub-samples from each vial were analysed three times each under repeatability conditions. All samples were analysed randomly to ensure that the order of measurements did not correspond to the filling sequence of the vials. Data were analysed by ANOVA to quantify homogeneity of samples and estimation of uncertainty due to heterogeneity effect.

2.5. Stability Testing

Ledipasvir and sofosbuvir pure materials were submitted to isochronous stability studies to investigate stability of materials under the storage and transport conditions [26-30]. Random sampling
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approach was applied to select twenty-five vials from each patch, the vials were divided into four groups, and each group consisted of six vials. The groups were stored at 4 °C and 40 °C, two groups at each temperature and one vials were stored at reference temperature (-20 °C). One sample from each group at the two-storage temperature was moved to reference temperature after 4, 8, 12, 16, 20 and 24 months for the long-term stability study and 1, 2, 3, 4, 5 and 6 weeks for the short-term stability study, the vials that initially stored at the reference temperature used to estimate the starting value for t=0. At the end of study, all vials were analysed in duplicates under repeatability conditions in one batch. The data were statistically analysed for outliers and regression analysis as a function of time was performed and slopes were tested for significance using a t-test [20, 21].

3. Results and discussion

3.1 Characterization of Materials

The identity of sofosbuvir and ledipasvir was confirmed by their mass spectra and UV-VIS spectral characteristics using LC-MS/MS and HPLC-DAD, respectively. The MS/MS was operated at negative electrospray ionization mode (ESI-) and collision energy of 2V, ionization was achieved using water with 0.1% formic acid and acetonitrile as mobile phase at a flow rate of 0.2 mL/min. MRM chromatogram of sofosbuvir (Figure S1, see supporting information) show absence of any organic impurity components with purity of 100 %, where two significant organic impurities were detected in ledipasvir material (Figure S2, see supporting information) at retention times 9.17 and 12.22 with peak area 0.51 % and 0.61 % of total peak areas, respectively. Additionally, Figure S1 show mass spectra with the characteristic molecular ions of sofosbuvir and ledipasvir with m/z values of 528.2344 and 887.6618 (\([M-H]\)-), respectively. On the one hand, HPLC-DAD was used for obtaining the characteristic UV-VIS absorption for each analyte in the range 190 to 1100 nm, the UV characteristics illustrated in Figure S3 (see supporting information) and Figure S4 (see supporting information) shows absorption maximum at 260 nm and 330 nm for sofosbuvir and ledipasvir, respectively.

On the one hand, the quantitative determination of structurally related impurities was based on RP-HPLC-DAD operated at a wavelength of 260 and 330 nm for sofosbuvir and ledipasvir materials, respectively. The ledipasvir and sofosbuvir peaks were well resolved without interferences by related materials with retention times of 18.97 and 18.85 min, respectively. Representative HPLC chromatograms for both Ledipasvir (Figure 3) and sofosbuvir (Figure 4) shows very sharp peaks for both analytes with organic impurities peaks. Figure 3 show three peaks detected at 15.207, 16.022 and 17.620, and comprised 0.102 % of the total chromatogram peak area, where Figure 4 show six peaks detected at 12.923, 16.888, 17.528, 20.841, 23.085 and 23.309, and comprised 0. 0.085 % of the total chromatogram peak area. The mean chromatographic purity of ledipasvir and sofosbuvir determined by an area normalization method according to Eq. 1 was 99.898 and 99.915 %, respectively.

\[
\text{Chromatographic Purity} = \frac{A_{\text{Analyte}}}{A_{\text{Analyte}} + \sum_{i=1}^{n} A_{\text{impurity},i}}
\]  

Where, \(A_{\text{Analyte}}\) and \(A_{\text{impurity},i}\) are peak areas of analyte (main peak) and impurity i, respectively.

3.1.1 Determination of Volatile Organic Compounds (VOCs)

A trace level of iso-propanol was detected in ledipasvir by headspace GC–FID and DB-VRX column. Figure S5 (see supporting information) show very sharp peaks at retention time of 10.73 min, the base line show no other residual solvents or VOCs contained in the ledipasvir material. Six vials were selected from each batch, the whole content was transferred directly to headspace test vials without any treatments to avoid contamination or analyte loss. Iso-propanol was the only detected volatile residues in ledipasvir materials with concentration of 0.0060 %, where these analytes were not detected in sofosbuvir material.
3.1.2 Determination of Inorganic Impurities

Inductively coupled plasma optical emission spectrometer (ICP-OES) and ion chromatograph were used for determination of inorganic impurities including trace elements and anions, respectively. For trace elements analysis, 0.1 g of sample was digested with 25 ml of nitric acid (80 % (v/v)), the samples were directly measured in triplicates. ICP-OES results indicated that the ledipasvir material contains Zn, Fe, Cr and Al with concentrations of 0.00015 %, 0.00006 %, 0.00010 %, and 0.00004 %, respectively. Where, sofosbuvir material contains Zn, Fe and Cr with concentrations of 0.00070 %, 0.00008 %, and 0.00002 %, respectively.

3.1.3 Determination of Water Content

The water content in the prepared materials was determined using coulometric Karl Fischer titrator equipped with diaphragmed cell generator and 10 µA polarization current, the endpoint was detected automatically at stop drift of 10 µg/min. About 100 mg samples were accurately weighed and transferred to titration cell. The samples were measured in triplicate, and mean values were 0.205 %, and 0.118 % for ledipasvir and sofosbuvir, respectively. The water content represents the highest impurity content in both materials.

Table 1. Results of homogeneity study for ledipasvir and sofosbuvir reference materials

<table>
<thead>
<tr>
<th>Material</th>
<th>Source of Variation</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit.</th>
<th>Uncertainty (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sofosbuvir</td>
<td>Between vials</td>
<td>5.576148</td>
<td>0.534131</td>
<td>0.832668</td>
<td>2.392814</td>
<td>0.000192</td>
</tr>
<tr>
<td></td>
<td>Within vials</td>
<td>10.43967</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ledipasvir</td>
<td>Between vials</td>
<td>3.840926</td>
<td>0.981664</td>
<td>0.483833</td>
<td>2.392814</td>
<td>0.00024</td>
</tr>
<tr>
<td></td>
<td>Within vials</td>
<td>3.912667</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For anions, the simple and efficient ion chromatograph method was used to detect fluoride, chloride, bromide, nitrate, sulphate, and phosphate potentially present in both materials. Three vials were selected randomly from each material and three portions from each vial were directly measured in triplicates. The six anions were not detected in both materials. Based on these results, the detected trace elements are present in the materials in the elemental state and total amount of inorganic impurities are 0.00017 % and 0.00080 % for ledipasvir and Sofosbuvir, respectively.

3.2. Homogeneity Study

The homogeneity of the ledipasvir and sofosbuvir batches were fully assessed by analysis of about 10 % of prepared vials, three independent sub-samples from each vial were analysed by HPLC method three times each under repeatability conditions. The data from homogeneity study were analysed for outliers by Grubb’s test and significant differences in the purity graphically (Figure 1, S1, L1) and statistically using ANOVA statistical tool in Minitab 20 software. ANOVA table for each analyte was generated and calculated values of the F-distribution were compared with tabulated values at 95 % significance level. The P-value was considered to be statistically significant when P-value < 0.05. According to the homogeneity test results illustrated in Table 1, no statistically significant variability was found between vials based on P-value and F test. P-value is higher than 0.05 and F distribution is smaller than F critical for both analytes. The mean squares MS<sub>between</sub> and MS<sub>within</sub> from ANOVA table were used to estimate uncertainty due to materials heterogeneity (u<sub>bb</sub>) using equation 1.

\[
u_{bb} = \sqrt{MS_{within} \cdot \ln \frac{\chi^2(v)}{\sqrt{vMS_{within}}}}
\]

Where MS<sub>between</sub> represents between groups mean squares, MS<sub>within</sub> represents within groups mean squares and v is the degree of freedom of MS<sub>within</sub>. The estimated uncertainty was combined with
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uncertainties from other sources to estimate expanded uncertainty associated with reference value of each analyte [30-32]. The small values of uncertainty due to materials heterogeneity (\(u_{\text{mb}}\)) revealed a high degree of concordance among the prepared vials. The batches under certification had good agreement between vials for both analytes and, it was considered as sufficiently homogeneous.

**Figure 1.** S1, L1, S2, L2, S3, and L3: Graphical representation of homogeneity assessment and linear regression plots for long-term stability study during 24 months of storage at 4 and 40 °C of sofosbuvir and ledipasvir, respectively.

### 3.3. Stability Study

Stability studies are conducted to investigate short- and long-term stability and to establish both dispatch and storage conditions. Isochronous approach was applied to evaluate to types of stability, the vials were stored for 6 weeks and 24 months at 4 °C and 40 °Cand reference vials at -20 °C. After the
predetermined storage periods, the samples were returned and stored at the reference temperature (-20 °C), all samples were analyzed after 24 months in the same time in duplicates under repeatability conditions in one batch. With this procedure the risk of having deviations in the response of the HPLC instrument are minimized. The data were collected and screened for single and double outliers by applying the Grubbs test at confidence levels of 95 and 99 %, respectively. The resulting peak areas were plotted as a function of time (Figure 6, S2, S3, L2, and L3) and the regression line was checked for significant trends by t-test statistics. Figure 6, S2, L2 showed no significant degradation at 4 ℃ for both materials, but a significant degradation was observed for materials at 40 ℃ (Figure 6, S3, and L3). Therefore, storage temperature was defied in the materials certificate as ≤4 ℃. The short-term stability study evaluation showed that no significant degradation was detected for transport conditions at 4 ℃ and 40 ℃, the material can be transported at both conditions but dispatch and transportation at ≤4 ℃ was recommended for more protection of materials. Regression results for long-term stability study were used for estimation of shelf life and uncertainty due to materials instability according to the Eq. 3 [30-33].

\[ u_{lsx} = Y_0 X u_b \]  

(3)

Where, \( Y_0 \) is initial value, \( X \) is time point and \( u_b \) is standard uncertainty due to long term stability.

Estimated uncertainty was included in the combined uncertainty of the assigned value. The prepared CRMs are considered stable for 24 months, at the storage condition of ≤4 ℃. Stability testing will be continued over the period of material availability to maintain confidence in the certified values.

### Table 2. Measurements results and uncertainties of detected impurities in ledipasvir and sofosbuvir materials

<table>
<thead>
<tr>
<th>Test</th>
<th>Sofosbuvir</th>
<th>Uncertainty</th>
<th>Ledipasvir</th>
<th>Uncertainty</th>
</tr>
</thead>
<tbody>
<tr>
<td>Related materials impurities (%)</td>
<td>0.08500</td>
<td>0.00250</td>
<td>0.10200</td>
<td>0.00290</td>
</tr>
<tr>
<td>VOCs and residual solvents (%)</td>
<td>ND</td>
<td>0.0</td>
<td>0.00600</td>
<td>0.00090</td>
</tr>
<tr>
<td>Zn (%)</td>
<td>0.00070</td>
<td>0.00018</td>
<td>0.00015</td>
<td>0.00028</td>
</tr>
<tr>
<td>Fe (%)</td>
<td>0.00008</td>
<td>0.00002</td>
<td>0.00006</td>
<td>0.00001</td>
</tr>
<tr>
<td>Cr (%)</td>
<td>0.00002</td>
<td>0.00001</td>
<td>0.00010</td>
<td>0.00002</td>
</tr>
<tr>
<td>Al (%)</td>
<td>ND</td>
<td>0.0</td>
<td>0.00004</td>
<td>0.00001</td>
</tr>
<tr>
<td>Anions</td>
<td>ND</td>
<td>0.0</td>
<td>ND</td>
<td>0.0</td>
</tr>
<tr>
<td>Water contents (%)</td>
<td>0.12000</td>
<td>0.01100</td>
<td>0.21000</td>
<td>0.01800</td>
</tr>
<tr>
<td>Sum of impurities</td>
<td>0.20580</td>
<td>0.31835</td>
<td>0.31835</td>
<td></td>
</tr>
<tr>
<td>( u_{ch} ), Characterization uncertainty</td>
<td>0.01300</td>
<td>0.01870</td>
<td>0.01300</td>
<td>0.01870</td>
</tr>
<tr>
<td>( u_{bb} ), Uncertainty due to</td>
<td>0.00019</td>
<td>0.00024</td>
<td>0.00019</td>
<td>0.00024</td>
</tr>
<tr>
<td>( u_{bs} ), Uncertainty due stability (%)</td>
<td>0.00014</td>
<td>0.00038</td>
<td>0.00014</td>
<td>0.00038</td>
</tr>
<tr>
<td>( u_c ), Combined standard uncertainty (%)</td>
<td>0.014</td>
<td>0.019</td>
<td>0.014</td>
<td>0.019</td>
</tr>
<tr>
<td>Certified value (%)</td>
<td>99.79</td>
<td>99.68</td>
<td>99.79</td>
<td>99.68</td>
</tr>
<tr>
<td>( U_{exp} ), Expanded uncertainty (%)</td>
<td>0.03</td>
<td>0.04</td>
<td>0.03</td>
<td>0.04</td>
</tr>
</tbody>
</table>

3.4. Value Assignment and Uncertainty Budget of Reference Values

The mass balance approach was applied for purity determination, all detected impurities mass fractions were transformed to percentage unit (%) and combined to provide single quantitative estimate for all impurities [23-30]. The impurities estimate was subtracted from unity (100 % − ∑ % Impurities) and assigned value of sofosbuvir and ledipasvir was assigned to be 99.79 % and 99.69 %, respectively. The uncertainty of the certified value is propagated through each step of the measurement procedure and affects laboratory uncertainty budgets. So, the uncertainty of the certified value was estimated in
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accordance with ISO Guide 35 and GUM [30, 34-35] from the uncertainty components (Figure 2) due to impurities characterization ($u_{ch}$), sample inhomogeneity ($u_{bb}$) and long-term instability ($u_{ls}$). Uncertainty due to impurities characterization ($u_{ch}$) was estimated from measurement of heavy metals ($u_{hm}$), water content ($u_{w}$), residual solvents and VOCs ($u_{voc}$) and structurally-related organic impurities ($u_{oi}$).

![Figure 2. Fishbone diagram for uncertainty sources of sofosbuvir and ledipasvir RMs measurements.](image)

The expanded uncertainty ($U_{EXP}$) is expressed as two times the root of the sum of the squares of $u_{ch}$, $u_{bb}$ and $u_{ls}$ at a confidence level of approximately 95% [30, 34-35], the certified values (Table 2) and uncertainties of sofosbuvir and ledipasvir were found (99.79±0.03) %, (99.69±0.04) %, respectively.

4. Conclusions

The purity of HCV sofosbuvir and ledipasvir drugs was indirectly assessed through determination of mass fractions of anions, heavy metals, water content, residual solvents, and VOCs, as well as structurally related organic impurities. The impurities mass fractions in each material were assigned by validated chromatographic methods (IC, HPLC-DAD, LC-MS/MS, HS-GC, GC-MS), Karl Fisher titrator, and inductively coupled plasma-based method. The identity of both drugs and structurally related impurities were identified by liquid chromatography mass spectrometer but quantification of these impurities was based on HPLC-DAD measurements. The stability of materials was evaluated for two years, and homogeneity assessment was based on study of about 10% of prepared vials. The certified value was estimated by subtracting mass fraction of combined estimate of all detected impurities from unity. The expanded uncertainty of the certified value was based on uncertainty of characterization, homogeneity, and stability of each material. The certified purity values of sofosbuvir and ledipasvir and their corresponding expanded uncertainties ($k = 2.0$ at 95% CL) were found (99.79±0.03) %, (99.69±0.04) %, respectively. The developed certified reference materials (CRMs) with small uncertainty values will be valuable tool for supporting pharmaceutical testing laboratories in their efforts to maintain and improve quality of results and provide them with high order CRMs for the accurate determination of both analytes in raw materials and finished products.

Supporting Information

Supporting information accompanies this paper on [http://www.acgpubs.org/journal/journal-of-chemical-metrology](http://www.acgpubs.org/journal/journal-of-chemical-metrology)
References


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