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# A validated high performance liquid chromatographic method for analysis of isosorbide mononitrate in bulk material and extended release formulations

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**Abstract**

A reversed phase HPLC method using $C_{18}$ column was developed for the quantitative determination of isosorbide mononitrate (IMN) in the bulk material and extended release dosage forms. The method was specific to IMN and able to resolve the drug peak from the pharmacopoeial impurities and formulation excipients. The method was accurate, precise, and linear within the desired range. In addition to analysis of assay and dissolution samples, the method was successfully used for analysis of drug–excipient compatibility samples of IMN used for development of extended release formulations in our laboratory and their subsequent stability studies. The method was also used for analysis of IMN in commercially available raw material. 

*Keywords:* Isosorbide mononitrate (IMN); Validation; Extended release; Dissolution; Stability studies; Compatibility studies

## 1. Introduction

Isosorbide 5-mononitrate (IMN) belongs to the category of organic nitrates and is mainly indicated for the treatment of conditions such as stable and unstable angina pectoris, acute myocardial infarction, and heart failure [1]. It is a major metabolite of isosorbide dinitrate (ISDN) and offers several therapeutic advantages over other organic nitrates, such as good oral absorption, long elimination half-life (4–5 h) in comparison to ISDN [2], and absence of first pass metabolism [3]. It is highly soluble (see Section 2) and highly permeable (absolute bioavailability $>90\%$) [4] and thus belongs to class 1 according to Biopharmaceutics Classification System [5].

A number of methods have been used for analysis of IMN in bulk drug as well as pharmaceutical dosage forms such as polarographic [6], DSC [7], GC [8], TLC [7,9], and HPLC [7,10–14]. As a part of development of extended release formulations of IMN in our laboratory, HPLC method using a $C_{18}$ column was developed and validated for analysis of assay and dissolution samples of IMN. The advantage with the proposed method is

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that a single method can be used for assay and dissolution studies. In addition, the method has been successfully used for analysis of drug–excipient compatibility samples of IMN during the formulation development and subsequent stability studies of *in house* formulations. The method has also been used for analysis of IMN in commercially available bulk material.

## 2. Experimental

### 2.1. Instrumentation

A Shimadzu HPLC system equipped with LC-10 AT VP pump, DGU-14 AM on-line degasser, SIL-10 AD VP autoinjector, CTO-10 AS VP column oven, and SPD-10 A VP UV–VIS detector was utilized. The second instrument used for peak purity testing was a Shimadzu HPLC system equipped with LC-10 AT VP pump, DGU-14 AM on-line degasser, Rheodyne manual injector fitted with a 20 μl loop, CTO-10 A VP column oven, and a SPD-M 10 A VP PDA detector. Shimadzu CLASS-VP software (Version 5.03) was used for data acquisition and mathematical calculations. The chromatographic conditions are outlined in Table 1. In addition, Mettler Toledo AG-245 electronic balance, Branson 3510 ultrasonic water bath, Microlitre syringe from Hamilton, and Millipore filtration assembly were used in this study. Water used throughout the HPLC analysis was prepared by reverse-osmosis using USF ELGA machine. Dissolution studies were conducted in USP 23 dissolution apparatus (Electrolab, India) and samples for stability studies were kept in ICH certified stability chambers (WTC Binder, Germany) maintained at 40 °C and 75% RH.

Table 1
HPLC parameters for determination of IMN

| Parameter | Condition |
|---|---|
| Method | Reversed phase high performance liquid chromatography |
| Mobile phase | Isocratic elution, Water-Methanol (80:20, v/v) |
| Column | $C_{18}$ Spherisorb (Waters, USA), 4.6 × 250 mm and 5 μm particle size |
| Flow rate | 1 ml/min |
| Detection | UV detector, 220 nm<br>PDA detector, 200–800 nm for peak purity testing |
| Column temperature | 25 °C |
| Injection volume | 20 μl for assay and compatibility samples<br>50 μl for dissolution samples |

### 2.2. Materials

IMN (99.9% purity) was a gift sample from JP Fine Chemicals, India. Working standards of IMN (99.8% purity), ISDN (100.2% purity), and isosorbide 2-mononitrate (2-IMN, 99.3% purity) were obtained as gift samples from Sifa Chemicals, Switzerland. Three different extended release formulations of IMN namely Imdur (Astra IDL Ltd., India), Angicor (Novartis India Ltd., India), and Angitab (ICI India Ltd., India) were obtained from retail pharmacies. Each product was labeled to contain 60 mg of IMN. The details of extended release formulation (containing 60 mg of IMN) developed in our laboratory (NIPER formulation, NIPER 1-R-3) are given elsewhere [15]. IMN bulk material (average potency ranging from 80 to 100%) was obtained as a gift sample from three different companies (JP Fine Chemicals, India; Nicholas Piramal India Limited, India; and Recon Limited, India). For the purpose of this work, identity of the manufacturers is not disclosed and the bulk materials are coded. All the excipients used for the development of NIPER formulations were obtained from commercial sources and were used as such. Methanol used was of HPLC grade (Rankem, India) and water was obtained by reverse osmosis.

### 2.3. Preparation of stock solutions

An accurately weighed amount (approximately 62.5 mg) of IMN was added into a 250 ml volumetric flask. The sample was dissolved in methanol to give a concentration of approximately 250 μg/ml. This solution was labeled as standard stock solution. An accurate amount (approximately 6.5 mg) of 2-IMN and ISDN were

separately dissolved in 25 ml of methanol to give concentration of approximately 260 μg/ml.

### 2.4. Method validation

The method was validated for the parameters like specificity, range and linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy, and precision. In addition, stability of the analytical solutions was determined and system suitability parameters were also calculated.

To determine the specificity of method in the presence of pharmacopoeial impurities, 5 ml solution of IMN, ISDN, and 2-IMN (having a concentration of approximately 250 μg/ml each) was added into a 25 ml of volumetric flask and volume made up to 25 ml with mobile phase. The resulting solution contained approximately 50 μg/ml of each and was used for specificity studies. To demonstrate specificity in the presence of excipients used in formulation development, IMN was spiked in the placebo (at approximately 100 μg/ml level) and chromatogram observed. The purity of the peak was also checked using a photo-diode array (PDA) detector.

To evaluate the linearity of the method, different dilutions were made from the standard stock solutions in the working range of 80–120 μg/ml (with mobile phase), 0.5–80 μg/ml (with simulated intestinal fluid), and 15–75 μg/ml (with mobile phase) for assay, dissolution, and compatibility studies, respectively. Five standard solutions were analyzed in case of assay and compatibility studies and seven for dissolution studies.

To determine the LOD and LOQ, serial dilutions of IMN, 2-IMN, and ISDN were made from the standard stock solution in the range of 200–750 ng/ml. The samples were injected (20 μl) and measured signal from the samples was compared with those of blank samples. LOD and LOQ values were identified as signal-to-noise ratio of 3:1 and 10:1, respectively.

To determine accuracy of the method, working standard of IMN was prepared in triplicate at three concentration levels (80, 100, and 120 μg/ml for assay and 5, 35, and 65 μg/ml for dissolution studies) and analyzed. Samples for recovery studies were prepared by spiking known amount of drug in the placebo at three concentration levels (80, 100, and 120 μg/ml).

Repeatability of the method was checked by analyzing six replicate samples of IMN (at the 100% concentration level) and calculating relative standard deviation (%RSD). To determine intermediate precision, standard solutions of IMN at five concentration levels were analyzed three times within the same day (intra-day variation) and on three different days (inter-day variation).

To check the solution state stability of IMN, standard solutions of IMN (10 and 120 μg/ml) were prepared in simulated intestinal fluid and mobile phase and kept in refrigerator and at 37 °C. The samples were analyzed next day against a freshly prepared standard.

Data from six replicate injections (at 100% test concentration) was utilized for calculating various system suitability parameters using Shimadzu CLASS-VP software.

### 2.5. In vitro analysis

#### 2.5.1. Assay

In case of marketed formulations, five accurately weighed tablets were crushed to a fine powder and an amount equivalent to 10 mg of IMN was added into five different 100 ml volumetric flask and volume made up with mobile phase. The samples, after filtration through a 0.45-μm nylon membrane filter, were injected for HPLC analysis. The ingredients used in the NIPER 1-R-3 formulation were sticky and to avoid any non-uniformity during sampling, one accurately weighed tablet ($n = 5$) was dissolved in 250 ml of water. The samples, after filtration through a 0.45-μm nylon membrane filter and dilution with the mobile phase (10 ml of the filtrate up to 25 ml), were used for HPLC analysis.

#### 2.5.2. Dissolution studies

Dissolution studies of the marketed and NIPER formulations ($n = 6$ in each case) were carried out using USP-I dissolution apparatus using rotating basket method (100 RPM). The drug was highly soluble as determined from the

experimental studies (highest dose strength soluble in $<250$ ml of aqueous media over the pH range of 1–7.5 at 37 °C). Simulated Intestinal Fluid, pH 6.8 (900 ml) maintained at $37 \pm 0.5$ °C was used as dissolution medium. The samples (10 ml) were withdrawn at predetermined time and replaced with an equivalent amount of fresh medium. The samples were filtered through a 0.45-μm nylon membrane filter and analyzed. The cumulative percent drug release was plotted against time to determine the release profile.

*2.5.3. Drug–excipient compatibility analysis*

Technique of isothermal stress testing was utilized for assessing the compatibility of IMN with the excipients used in the formulation development [16]. IMN and different excipients (in the ratio similar to that expected in the final formulation) were weighed directly in 4-ml glass vials ($n = 2$) and mixed on a vortex mixer for 2 min. In each of the vials, 10% w/w water was added and the drug–excipient blend was further mixed with a glass capillary (both the ends of which were heat sealed). To prevent any loss of material, capillary was broken and left inside the vial. Each vial was sealed using a Teflon-lined screw cap and stored at 50 °C. After 3 weeks of storage at the above conditions, samples were quantitatively analyzed. Drug–excipient blends without added water and stored in refrigerator served as controls. For sample preparation, 2 ml of mobile phase was added to each vial. The mixture was vortexed and transferred to 100 ml volumetric flask. Vials were rinsed twice with the mobile phase to dissolve the residual amount. Mobile phase was added to the volumetric flask and after sonication; volume was made up with mobile phase. The samples were centrifuged and the supernatant was filtered through a 0.45-μm nylon membrane filter. After appropriate dilutions, samples were analyzed and drug content was determined from the calibration curve prepared within the expected range.

*2.5.4. Stability studies*

NIPER 1-R-3 formulations were packed in 0.04 mm thick aluminum foil laminated with PVC coating and stored in ICH certified stability chambers maintained at 40 °C and 75% RH. The samples were withdrawn periodically (0–3 months) and subjected to assay and dissolution studies as per the procedure mentioned above.

*2.5.5. Determination of potency of raw material*

The potency of commercially available raw material of IMN was determined against the working standard. Drug was weighed and diluted to give concentration within the desired range (80, 100, and 120 μg/ml). The potency was calculated by comparing the response (area) of the sample with the response of the working standard (at the similar concentration level).

## 3. Results and discussion

### *3.1. Method validation*

*3.1.1. Specificity*

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present in the sample matrix [17]. For the specificity determination, both the impurities that are official in European and British Pharmacopoeia (ISDN and 2-IMN) were added to the pure IMN sample. The representative chromatogram showing the resolution between IMN and its closely eluting impurity (2-IMN) is given in Fig. 1. Retention time of ISDN was found to be 40.18 min and it does not interfere with the drug peak.

For demonstrating the specificity of the method for drug formulation (*in house*), mixture of IMN with all the excipients was prepared and the representative chromatogram is shown in Fig. 2. The excipients used in the formulation did not interfere with the drug peak and thus, the method is specific for IMN. To further confirm the specificity of the method, UV scans of excipients were taken (200–400 nm) and none was found to absorb significantly at the analytical wavelength of drug (220 nm). To demonstrate the method's specificity for marketed formulations, peak purity testing using the PDA detector was performed. The purity of the peak was found to be 100%, demonstrating that the drug peak is attributable only to a single component (IMN).

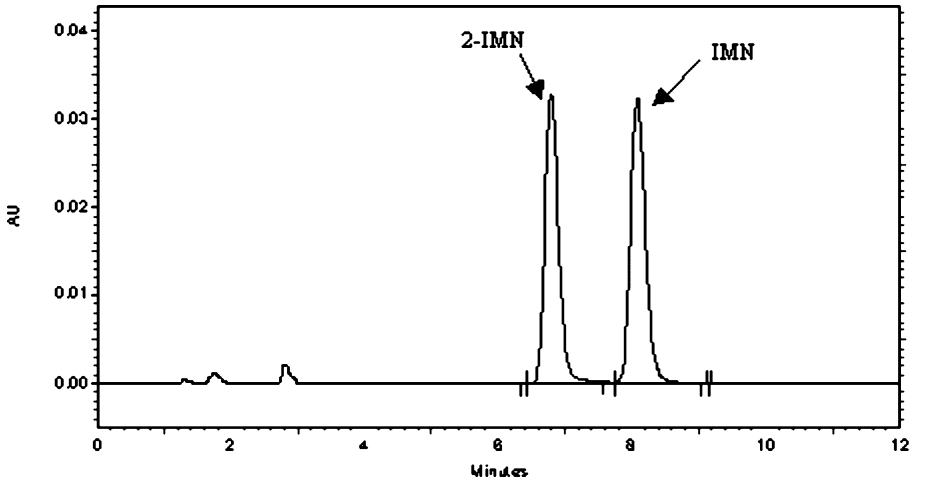


Fig. 1. Chromatogram showing resolution of IMN and its closely eluting impurity 2-IMN. The resolution obtained between IMN and 2-IMN is 3.20.

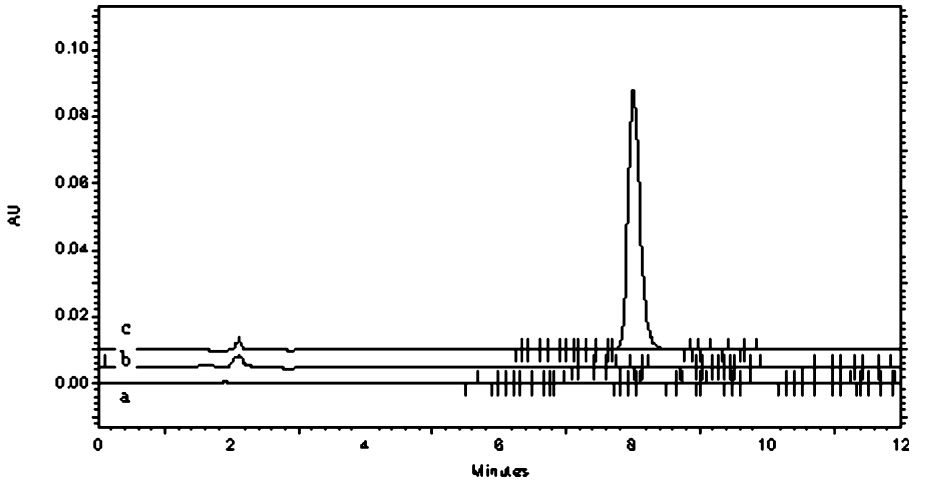


Fig. 2. Overlay of chromatograms of blank (a), excipient blend (b), and IMN in the presence of all the excipients used in the formulation development (c).

### 3.1.2. Range and linearity

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range [17]. The linearity of the method was observed in the expected concentration range demonstrating its suitability for analysis. The regression statistics are shown in Table 2. The goodness-of-fit ($R^2$) was found to be $>0.99$ and value of intercept was less than 2% of the response of 100% of the test concentration in all the cases indicating functional linear relationship between the concentration of analyte and area under the peak.

### 3.1.3. Detection and quantitation limit

The detection limit or LOD is the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated, under the

stated experimental conditions. It may be expressed as a concentration that gives a signal to noise ratio of 2:1 or 3:1 [17]. The lower limits of detection for IMN, 2-IMN, and ISDN were found to be 250, 250, and 300 ng/ml, respectively. Quantitation limit or LOQ is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. A signal to noise ratio of 10:1 can be taken as LOQ of the method [17]. The LOQ values for IMN, 2-IMN, and ISDN were found to be 600, 500, and 600 ng/ml, respectively.

### 3.1.4. Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value [17]. It can be determined by application of the analytical procedure to an analyte of known purity (for the drug substance) or by recovery studies, where known amount of standard is spiked in the placebo (for the drug product). The results of accuracy studies are shown in Table 3, and it is evident that the method is accurate within the desired range.

### 3.1.5. Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of a homogenous sample [17]. Repeatability refers to the use of the analytical procedure within a laboratory over a short period of time using the same analyst with the same equipment and is expressed as the %RSD. The method passed the test for repeatability as determined by %RSD of the area of the peaks of six replicate injections at 100% test concentration (0.62 and 1.05% for assay and dissolution studies, respectively). The results of intra- and inter-day variation are shown in Table 4.

### 3.1.6. Solution state stability

To demonstrate that IMN does not degrade in the dissolution media (simulated intestinal fluid) during the test and in the HPLC autosampler (during the sequence run), solution state stability of IMN was carried out. The results demonstrated that there was no significant change in the drug content (drug content ranged from 98.72 to 100.40%) and the solutions were stable for at least 24 h. During the stability studies, no additional

Table 2
Regression statistics for analysis of IMN

| Parameter | Target concentration (μg/ml) | Range (μg/ml) | Goodness-of-fit ($R^2$) | Slope | Intercept |
|---|---|---|---|---|---|
| Assay | 100 | 80–120 | 0.9993 | 10187.67 | 18420.09 |
| Dissolution | 66.67 | 0.5–80 | 1 | 24883.44 | 11758.90 |
| Compatibility studies | 45 | 15–75 | 0.998 | 10 409 | 3861.9 |

Table 3
Accuracy/recovery data for IMN

| Parameter | Concentration (μg/ml) | IMN recovery | %RSD |
|---|---|---|---|
| Assay | 80 | 100.89 | 0.54 |
| | 100 | 100.88 | 0.27 |
| | 120 | 101.09 | 0.11 |
| Assay (spiking method) | 80 | 98.14 | 1.74 |
| | 100 | 97.93 | 1.32 |
| | 120 | 96.66 | 0.40 |
| Dissolution | 5 | 90.29 | 1.91 |
| | 35 | 104.64 | 0.69 |
| | 65 | 102.36 | 0.38 |

Table 4
Intermediate precision of the method

| Assay | | | Dissolution | | |
|---|---|---|---|---|---|
| Concentration (μg/ml) | Intra-day variation (%RSD) | Inter-day variation (%RSD) | Concentration (μg/ml) | Intra-day variation (%RSD) | Inter-day variation (%RSD) |
| 80 | 0.56 | 0.92 | 5 | 2.29 | 2.38 |
| 90 | 0.13 | 1.23 | 35 | 0.85 | 1.80 |
| 100 | 0.57 | 0.68 | 50 | 0.52 | 1.85 |
| 110 | 0.34 | 0.70 | 65 | 0.75 | 0.14 |
| 120 | 0.46 | 1.05 | 80 | 0.57 | 0.60 |

Table 5
System suitability parameters

| Parameter | Minimum | Maximum | Average | %RSD |
|---|---|---|---|---|
| Capacity factor ($K'$) | 3.56 | 3.59 | 3.57 | 0.316 |
| Number of theoretical plates ($N$) | 7541.17 | 7669.58 | 7604.72 | 0.621 |
| Tailing factor ($T$) | 1.25 | 1.25 | 1.25 | 0.051 |
| Retention time (min) | 7.977 | 8.027 | 8.005 | 0.247 |
| Area | 994 506 | 1 016 381 | 1 007 768 | 0.802 |

Table 6
Assay of IMN in marketed and NIPER formulations

| Serial number | Product | IMN label claim (mg per tablet) | Amount found (mg) | Recovery (%) | %RSD |
|---|---|---|---|---|---|
| 1 | Imdur | 60 | 64.13 | 106.88 | 0.84 |
| 2 | Angicor | 60 | 62.36 | 103.93 | 5.07 |
| 3 | Angitab | 60 | 63.29 | 105.49 | 1.25 |
| 4 | NIPER 1-R-3 | 60 | 60.56 | 100.94 | 4.52 |

peaks developed, and no changes in the chromatographic pattern was observed in either of the solutions.

#### 3.1.7. System suitability testing

System suitability tests are an integral part of chromatographic methods and are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. As seen from the Table 5, all the values for the system suitability parameters are within limits [18].

### 3.2. Applicability of method for IMN analysis

The developed method was applied for the determination of IMN content in marketed and NIPER extended release formulations. The assay results are shown in Table 6, demonstrating the suitability of method. Dissolution samples of IMN were also analyzed using this method. Drug release from the marketed and NIPER 1-R-3 formulations is shown in Fig. 3.

The method was also used for analyzing the samples of drug–excipient compatibility studies.

The excipients used in the development of NIPER formulation were tested for compatibility with the drug using the technique of isothermal stress testing. The drug content after 3 weeks of storage at the stress conditions was found to be within 98.34–102.21%. No change in the chromatographic pattern was observed in either of the samples and it was concluded that the excipients selected are compatible with the drug.

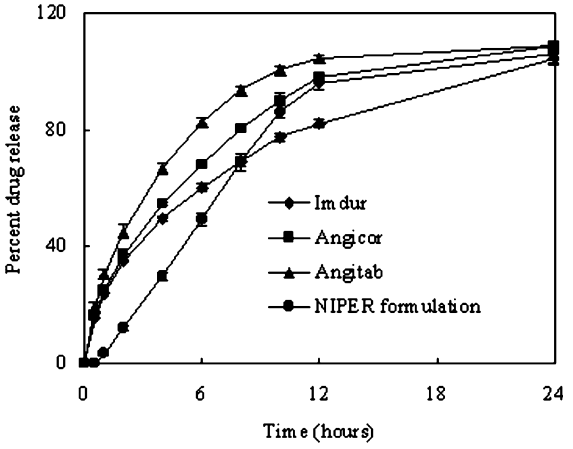


Fig. 3. Release profile of IMN from marketed and NIPER 1-R-3 formulations in simulated intestinal fluid, pH 6.8.

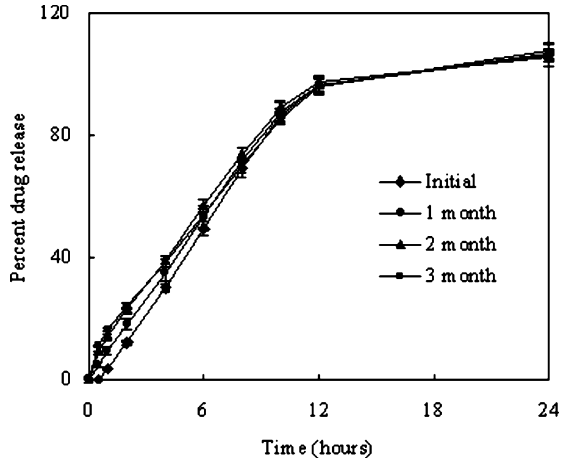


Fig. 4. Dissolution stability of NIPER 1-R-3 formulation after 3 months of storage at accelerated stability conditions (40 °C and 75% RH). The release profile is similar with respect to the initial sample as determined by the $f_2$ value (more than 50 in all case).

The method was successfully utilized for analyzing the stability samples of NIPER 1-R-3 formulations. The samples were withdrawn periodically and analyzed for drug content and dissolution stability. The samples were found to be stable as determined by the drug content (more than 90% in all the cases) and release profile (shown in Fig. 4). The release profile of the stability samples after 1–3 months was found to be similar with respect to the initial samples as determined by the $f_2$ value, which was more than 50 in all the cases [19]. The purity of the peak, as tested by PDA detector, was found to be 100%, demonstrating that the drug peak was attributable only to IMN.

The potency of commercially available raw material of IMN was also determined using this method. The potency of the raw material was calculated by comparing the area of the samples against that of a freshly prepared standard (at the similar concentration level). The results are shown in Table 7.

## 4. Conclusions

A simple HPLC method using a $C_{18}$ column was developed for analysis of IMN in bulk material and extended release formulations. This method was specifically developed for analysis of assay and dissolution samples of extended release formulations developed in our laboratory. The method was also successfully used for analysis of IMN in marketed formulations, analysis of drug–excipient compatibility samples for development of *in house* formulations, and their subsequent stability studies. The method can also be used for purity evaluation of IMN in the raw material. The developed method is specific to IMN, accurate, precise, and linear within the desired range.

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Table 7
Determination of potency of IMN raw material from different manufacturers

| Serial number | Company | Reported assay value (%) | Found assay value[a] (%) | Agreement (%) | %RSD |
|---|---|---|---|---|---|
| 1 | Manufacturer A | 100 | 98.26 | 98.26 | 0.823 |
| 2 | Manufacturer B | 80.1 | 79.48 | 99.22 | 0.685 |
| 3 | Manufacturer C | 80 | 75.92 | 94.90 | 0.956 |

[a] Average of three experiments.

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