Iranian Journal of Analytical Chemistry
Volume 2, Issue 2, September 2015 (112-119)

مجله ایرانی شیمی تجزیه
دوره ۲، شماره ۲، مهرماه ۱۳۹۴ (۱۱۹-۱۱۲)

نگرش HNMR برای ارزیابی ماده سمی N-Nدی متیل آمین در متفرمین هیدروکلرید: یک روش با کارآیی معادل نسبت به روش HPLC با مشتق سازی فاز معکوس

سیباپراساد ساهو[۱*]،نایسرگ دسای[۱]، آنیل بات[۱]، ک. شیراوامچاندرا[۱]، ب.و. کاماث[۲]
[۱]صنایع دارویی خورشید، تاندالجا، ودودارا، هند
[۲]انستیتو تکنولوژی، تحقیق و مدیریت، احمد آباد، هند
تاریخ دریافت: ۸ تیر ۱۳۹۴ تاریخ پذیرش: ۸ مرداد ۱۳۹۴

^{1}H qNMR Approach for Estimation of a Toxic Compound N,N-Dimethylamine in Metformin Hydrochloride: An Equivalent Efficient Method over Reversed Phase Derivatization HPLC Method

Sibaprasad Sahoo[1,*], Nisarg H. Desai[1], Anil Bhatt[1], K. Shivramchandra[1], B.V. Kamath[2]
[1] Sun Pharmaceutical Indusrties Ltd, Tandalja, Vadodara-390020, India
[2]Institute of Infrastructure, Technology, Research and Management, Ahmedabad-380008, India
Received: 29 June 2015 **Accepted:** 30 July 2015

چکیده
یک روش سریع، اختصاصی و دقیق بر اساس اسپکتروسکوپی روزنانس مغناطیسی هسته پروتون کمی به همراه روش کروماتوگرافی مایع با کارآیی بالا مشتقی فاز معکوس جهت اندازه گیری N,N دی متیل آمین هیدروکلرید در متفرمین هیدرو کلرید ارایه گردید. متفرمین هیدروکلرید ماتریکس دارویی فعال می باشد. در روش اسپکتروسکوپی روزنانس مغناطیسی هسته پروتون از گلیسین و دوتریم اکسید به ترتیب به عنوان استاندارد داخلی و رقیق کننده استفاده شد. هر دو روش به وسیله دستورالعمل های ICH اعتبار سنجی شدند و مشخص شد نتایج آنها قابل مقایسه می باشد و سودمندی های روش HNMR عبارتند از اینکه این روش اختصاصی و غیر تخریبی بوده و هیچ ماده مرجعی از N,N دی متیل آمین را نیاز ندارد. بنابراین این روش می تواند در تعیین N,N دی متیل آمین در نمونه دارویی متفرمین در خط تولید این دارو و همچنین در سایر محصولات دارویی تجاری مورد استفاده قرار گیرد.

واژه‌های کلیدی
متفرمین هیدروکلرید؛ N-Nدی متیل آمین؛ گلیسین؛ طیف سنجی ^{1}H qNMR.

Abstract
A rapid, specific and accurate proton quantitative Nuclear Magnetic Resonance spectroscopic (^{1}H qNMR) and also a reversed phase derivatization HPLC method have been developed to quantify a toxic, UV-visible inactive and non-volatile impurity N,N-Dimethylamine hydrochloride (DMA·HCl) in Metformin Hydrochloride (MF·HCl), an Active Pharmaceutical Ingradient. The method is based on proton quantitative NMR spectroscopy (^{1}H qNMR) using Glycine as internal standard and deuterium oxide (D_2O) as diluent. Both the methods have been validated as per the parameters of ICH guidelines and are found to be comparable.The advantages of the proposed ^{1}H qNMR method are that no certified reference standard of DMA·HCl is required for quantification, the method is specific, non-destructive and can be applied for quantification of DMA·HCl in process, quality control for the manufacturing of Metformin hydrochloride as well as commercial dosage form products.

Keywords
Metformin Hydrochloride; N,N-Dimethylamine Hydrochloride; Glycine; ^{1}H qNMR Spectroscopy.

1. INTRODUCTION

Metformin hydrochloride [1] 1,1-Dimethylbiguanide hydrochloride [2] is a biguanide hypoglycaemic agent commonly used for the treatment of type II diabetes [3]. Although MF·HCl was developed decades ago it is still used widely for the treatment of diabetes either alone or in combination with other drugs. From the analytical

*Corresponding Author: sibaprasad.sahoo@sunpharma.com

point of view several techniques [4-7] such as HPLC [8-9], Capillary Electrophoresis [10], NIR [11] and LC-MS-MS [12] for its determination in dosage form and biological fluids have been reported [13]. N,N-Dimethylamine hydrochloride (DMA·HCl) is a non volatile, colourless, crystalline secondary amine, which has low melting point. It is highly hygroscopic in nature and is also a precursor to several industrially significant compounds [14]. The solvents viz., dimethyl formamide (DMF) and dimethyl acetamide are derived from N,N-dimethyl amine hydrochloride. It is a raw material for the production of many agrochemicals, pharma-ceutical intermediates and finished products. The German cockroach utilizes dimethylamine as a pheromone for communication and DMA undergoes nitration under weak acid conditions to give dimethyl nitrosoamine. This animal carcinogen has been detected and quantified in human urine samples [15]. The manufacturing process of Metformin hydrochloride (MF·HCl), an active pharmaceutical ingredient (API), by using different routes of synthesis has always resulted into formation of a nonvolatile, UV-visible inactive (no chromophore) residual organic impurity [16-17], *viz* DMA·HCl, which is a residual solvent having specific higher limit by batch analysis data and the maximum daily dose capacity of MF·HCl pharmaceutical products. By considering the DMA·HCl as equivalent toxic solvent with organic residual impurity triethylamine, a permitted daily exposure (PDE) of 3.2 mg/day giving a limit of 320 ppm (for 10 g daily dose) was calculated for the organic base triethylamine from repeated dose toxicity and reproductive toxicity data [18]. In order to control the limit of DMA·HCl in Metformin hydrochloride below the PDE, there is a need for separate quantitative method which can directly identify and quantify it at ppm level. However, none of the reported methods for the determination of DMA·HCl in MF·HCl have been found to be stability indicating. Therefore, it is considered important to develop a stability indicating method, which can be employed in routine in-process and quality control analysis. (Fig. 1)

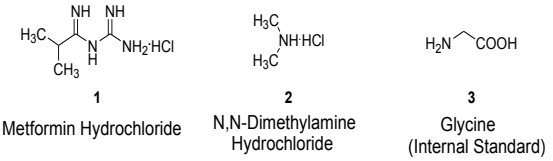

Fig. 1. Structure of drug substance (1), Analyte (2) and Internal standard (3).

Nuclear magnetic resonance (NMR) spectroscopy is a quantitative spectroscopic tool because the intensity of a resonance line is directly proportional to the number of resonant nuclei (spins). This fact enables accurate and precise determinations of the amount of substance needed. NMR has been used for quantitative determination of pharmaceutical compounds in different matrices. Quantitative measurement by NMR spectroscopy (^{1}H qNMR) was first described in 1963 for the determination of drugs [19-20].

Despite limited accuracy, proton quantitative NMR spectroscopy (^{1}H qNMR) finds application in various fields of science [21-31]. Lack of absorbing chromophores for UV-visible detection and the need for special chromatographic detectors as well as the difficulties in establishing highly efficient solid or liquid phase extraction procedures have made NMR a suitable alternative for biological sample analysis of many drugs [32-35]. To the best of our knowledge, no official method has been reported for the quantification of DMA·HCl by using ^{1}H qNMR. Hence the present study has been undertaken. The aim of this work is to develop advantageous and competitive selective proton quantitative NMR spectroscopic (^{1}H qNMR) method for the determination of DMA·HCl in in-process, quality control, API and in formulation samples that complies well with the validation requirements in the pharmaceutical industry. Apart from this, a new derivatization HPLC method [36-39] has also been developed. In ^{1}H qNMR method glycine has been used as internal standard (IS), by means of which simult-aneously 1,1-dimethyl hydrochloride (DMA·HCl) can be identified as well as quantified. This method is rapid, specific, simple and equivalent method to HPLC derivatisation method developed. Both the methods have been validated by assessing their specificity, linearity, range, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), ruggedness and robustness [40-41].

2. EXPERIMENTAL

2.1. Chemical and reagents

High purity analytical and ICH grade materials were used throughout the study. Metformine·HCl BP was provided by Sun Pharmaceuticals IndustriesLtd (Vadodara, India). Glycine was purchased from Merck, Germany (99.9 %). N,N-dimethylamine hydrochloride (DMA·HCl) was purchased from Aldrich, Germany (99.9 %). Deuterium Oxide (D_2O) diluent was purchased from Merck, Germany (99.96 % D).

The following materials were used in HPLC method: Acetonitrile HPLC grade (Merck), MilliQ water (from Milli-q system, Meck Millipore), Triethylamine HPLC grade (Merck), Orthophosphoric acid analytical reagent grade

(Merck) and 1-Fluoro-2,4-dinitro benzene (FDNB) analyical reagent grade (Merck).

2.2. ^1H qNMR experiments and methodology

All the ^1H qNMR spectra were recorded on a Bruker BioSpin AV-III 500MHz (11.7 T) spectrometer operating at proton frequency 500.13 MHz., using 5 mm Broad Band Observe probehead. All data were processed using Bruker`s Topspin 2.1 software. For all ^1H-NMR measurements carried out for method development, accurately 600 µL solution of 400 ppm concentration of DMA·HCl in pure D_2O solvent was taken in a 5 mm NMR tube. For generating ^1H NMR spectra, 16 scans were collected into 32 k data points over a spectral width of 9014.42 Hz., with a transmitter offset in the centre of the spectrum. The acquisition time was 9.49 min, followed by a relaxation delay of 35 s. All spectra were recorded at 295 K using a flip angle 90°. An exponential line broadening window function at 0.50 Hz was used in the data processing and baseline corrections were done automatically while phasing was always performed manully.

For accurate quantitative NMR analysis, the proper value of relaxation delay (d_1) was generally set at more than or equal to five times the value of the longest spin-lattice relaxation time (T_1) of the integrated resonance signals in order to ensure full relaxation of the corresponding protons. The T_1 relaxation time of MF·HCl, DMA·HCl and IS were measured by using NMR inversion-recovery pulse sequence, the maximum T_1 relaxation time values so obtained were maximum with 6.80s for DMA·HCl protons at δ 2.6 ppm, where as MF·HCl having methyl and glycine having methylene protons, T_1 relaxation time was 2.96 s at δ 2.92 ppm and 3.90s at δ 3.43 ppm, respectively. (Fig. 2)

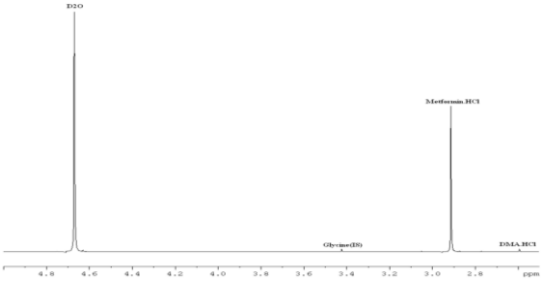

Fig. 2. NMR spectrum of MF·HCl with DMA·HCl along with Internal Standard (IS) as Glycine in D_2O.

2.3. Sample preparation for ^1H qNMR method

Diluent: Deuterium oxide (D_2O)

Stock solution (Internal Standard solution): Glycine (IS), 125 mg was taken in a 250 mL volumetric flask and volume was made up with diluent. Standard solution: DMA.HCl, 4 mg was dissolved in 1 mL of IS solution.

Sample preparation: 100 mg of Metformin.HCl was accurately weighed and dissolved in 5 mL of IS.

2.4. Chromatographic conditions

The quantitative derivatization gradient HPLC method for analysis of DMA·HCl in MF·HCl was performed on a Waters 2695 separation module equipped with dual wavelength detector 2487, using Empower 2.0 software, with a reverse phase column packed with octadecyl silane (C_{18}) chemically bonded phase particles with following optimal chromatographic conditions: Two mobile phases [Mobile phase A: 0.1% v/v orthophosphoric acid in water (buffer solution); Moblile phase B: 100% acetonitrile] were employed to run a gradient condition from 40%B to 55%B in 10 min, from 55%B to 75%B in 1 min, stay at 75%B for 4 min, from 75%B to 40%B in 0.5 min and stay 40%B till 20 min. Other optimal chromatographic conditions are listed in Table 1. The method was validated in accordance with the International Conference on Harmonization (ICH) guidelines for validation of analytical procedures.

Table 1. Optimal Chromatographic condtions.

Aspect	Description
Detection wave length	380 nm
HPLC Column	Inertsil ODS 3V (150 x 4. 6 mm ,5µm) , Make-GL Science, Japan
Flow rate	1.5mL/min.
Injection volume	20µL

2.5. In-situ derivatization process for N,N-Dimethylamine hydrochloride

A solution of 1.0 g of 2,4-dinitrofluorobenzene and equimolar amount of N,N-dimethylamine hydrochloride in acetonitrile was heated at 60°C for 30 min. The formed intermediate mixture of N,N-dimethyl-2,4-dinitrobenzene was neutralized using triethylamine as base to form the N,N-dimethyl-2,4-dinitrobenzene (UV-Vis active), salt of triethylammonium chloride and triethylamm-onium fluoride (Fig. 3).

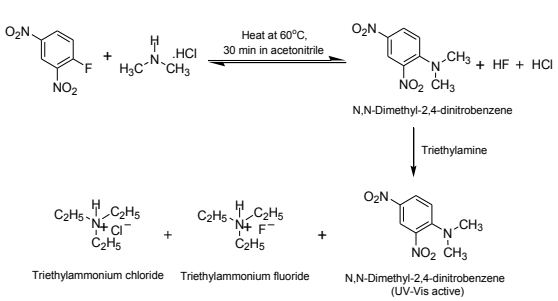

Fig. 3. Derivatization process for N, N-Dimethylamine hydrochloride (UV-Vis. active).

2.6. Standard and system suitability preparation

1-Fluoro-2,4-dinitro benzene (FDNB) solution was prepared by thoroughly mixing 1 mL of FDNB and small amount of acetonitrile solution in a 100 mL volumetric flask. This solution was thoroughly mixed and made up to the mark with acetonitrile and always prepared fresh just prior to use.

Dimethylamine hydrochloride (36.2 mg, equivalent to 20 mg of dimethylamine) was dissolved in a small amount of diluent in 100 mL volumetric flask and made upto the mark with the diluent. 2.5 mL of this solution was further diluted to 100 mL with the same diluent. 1 mL of this diluted solution was mixed thoroughly with 5 mL acetonitrile, 100 µL triethylamine and 1 mL of FDNB solution in 10 mL of volumetric flask. The mixture so prepared was heated at 60 ºC for 30 min and allowed to cool to attain room temperature and volume was made upto the mark with acetonitrile to give 0.5 ppm solution of dimethylamine.

2.7. Sample preparation

A 1000 ppm solution of MF·HCl was prepared by weighing 10 mg of the sample mixing thoroughly with 5 mL acetonitrile, followed by 100 µL triethyl amine and 1 mL of FDNB solution in 10 mL volumetric flask. The solution was heated at 60 ºC for 30 min and allowed to cool to attain room temperature and made upto the mark with acetonitrile. This solution was centrifuged at 3000 rpm for 5 min. in order to completely derivatize all DMA·HCl solvent present in MF·HCl.

3. RESULTS AND DISCUSSIONS

3.1 ^1H qNMR method development

^{1}H NMR spectrum of sample is shown in Fig.2. The signals at (δ) 3.4 ppm and 2.6 ppm (calibrated with chemical shift (δ) of HOD signal from D_2O at 4.6 ppm) are due to IS (Glycine) and N,N-Dimethylamine·HCl. The integral values of both the ^{1}H signals are found to be directly and quantitatively proportional to the number of nuclei and weight of the sample.

The DMA content was determined by using the Equation 1. given below.

$$W_x = \frac{I_x}{I_{std}} \times \frac{N_{std}}{N_x} \times \frac{M_x}{M_{std}} \times W_{std} \qquad (1)$$

where W_x = weight of DMA·HCl , I_x = area of DMA·HCl methyl signal, I_{std} = area of IS, N_{std} = number of nuclei involved in signal for standard, N_x = number of nuclei involved in signal for DMA·HCl, M_x is molecular weight of DMA·HCl , M_{std} is the molecular weight of IS and W_{std} weight of IS in sample solution. The ^{1}H qNMR method was validated in accordance with ICH gudelines through a study of linearity, method precision, LOD, LOQ, ruggedness, robustness, solution stability and recovery. The LOD and LOQ were calculated by the standard deviation of the response 'σ' and the slope 's' of the calibration curve (Fig. 3) obtained from the linearity results by using Equation $\text{LOD} = \frac{3.3\,\sigma}{S}$ and Equation $\text{LOQ} = \frac{10\,\sigma}{S}$, respectively.

3.2 ^1H qNMR method validation

3.2.1. Linearity

The linearity was evaluated and established by triplicate analysis of standard solution of DMA·HCl. The integrated NMR signal area obtained with respect to IS was plotted against the corresponding concentration to generate calibration curve (Fig. 4). Good linearity was evident (r^2 = 0.9991) over the examined concentration range of 1 ppm to 1000 ppm with equation y = 0.7524x ± 0.8303.

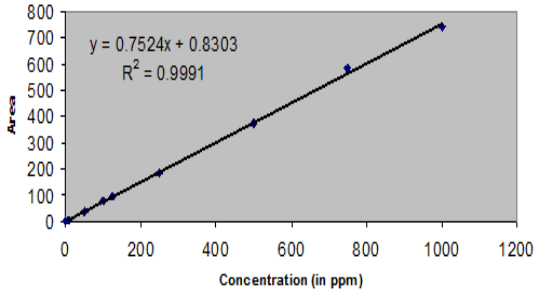

Fig. 4. Linearity plot of DMA·HCl content by ^{1}H qNMR.

3.2.2. Method precision, Ruggedness and Robustness study

Precision of the method, evaluated as repeatability, was studied by calculating the relative standard deviation (% RSD) of the peak areas of DMA·HCl for six determinations under the same experimental conditions. The %RSD of DMA·HCl integrated signal area obtained by ^{1}H qNMR method is 2.56%, which is well within the limit of not more than 10%. Ruggedness study was performed by applying analyst change, variation of sample depth, magnetic field (400 MHz instrument instead of 500 MHz instrument, Bruker Biospin AV-III, 400 MHz) and using different probe head (QNP-F), under the same acquisition parameters and data processing. The % RSD of DMA·HCl signal area obatained by ^{1}H qNMR method was 2.81% which is well within acceptable limit of not more than 10 %. Similarly, method robustness was determined by analyzing the same sample at normal operating conditions and also by changing some of the acquisition parameters of the instrument, *viz.,* number of scans (ns), recycle delay time(d_1), sweep width(sw), pulse width (p_1), off-set (o_1p), spinning rate (ro), and sample temperature. By changing only one operating parameter and keeping all the other acquisition parameters intact,

the sample was run for different number of scans and with optimum recycle delay, changing of sweep width, off-set value and spinning rate. In each case, the results obtained were close to the results obtained with normal instrumental condtions.

3.2.3. Accuracy and Recovery

According to ICH guidelines, additional proof of accuracy was obtained by three replicate determinations of three different concentration solutions of DMA·HCl in IS solution in presence of MF·HCl API containing 200ppm, 400ppm and 600ppm of DMA·HCl., corresponding to 50 %, 100% and 150% of the analytical concentrations, respectively. The obtained concentration values, when compared to the nominal values, produced accuracy of 101.5 %, 107.9 % and 111.7 % for the concentrations 50%, 100% and 150%, respectively, with an average %RSD value of 0.72 %, 2.51 % and 0.80 % respectively (Table 2, Table 3 and Table 4).

Table 2. Recovery at 50% level of DMA·HCl by ^{1}H qNMR

Sr. No.	I_x	W_x	% Recovery	Mean (%)	% RSD
1	215.00	1.95	100.82	101.5	0.72%
2	217.33	1.97	101.91	101.5	0.72%
3	217.00	1.97	101.74	101.5	0.72%

Table 3. Recovery at 100% level of DMA·HCl by ^{1}H qNMR

Sr. No.	I_x	W_x	% Recovery	Mean (%)	% RSD
1	420.67	3.81	109.37	107.9	2.51%
2	420.67	3.81	109.37	107.9	2.51%
3	404.33	3.66	105.02	107.9	2.51%

Table 4. Recovery at 150% level of DMA·HCl by ^{1}H qNMR

Sr. No.	I_x	W_x	% Recovery	Mean (%)	% RSD
1	618.00	5.60	110.80	111.7	0.80%
2	627.33	5.68	112.50	111.7	0.80%
3	623.33	5.64	111.77	111.7	0.80%

The results of the method were obtained within limit of accuracy 80 % to 120 % and % RSD not more than 10 %. Solution stability was checked for 96.0 hour by ^{1}H qNMR method (Table 5) and results were obtained to be very close to the content of DMA.HCl values obtained with freshly prepared sample.

3.2.4. Derivatisation HPLC method validation

The chromatogram of blank solution was obtained with run-time of 20 min as shown in Fig. 5. The peaks eluted at approximate retention times of 6.0, 9.0, 12.0, 13.0 and14.0 min are due to derivatized solutions, where as the peak with retention time of 7.4 min is due to DMA. (Fig. 6).

Table 5. Solution stability of DMA·HCl by ^{1}H qNMR analysis

Sr. No.	Time interval	W_x	Difference
1	0.0	3.25	N/A
2	12.0	3.24	0.01
3	24.0	3.23	0.02
4	48.0	3.24	0.01
5	72.0	3.22	0.03
6	96.0	3.21	0.04

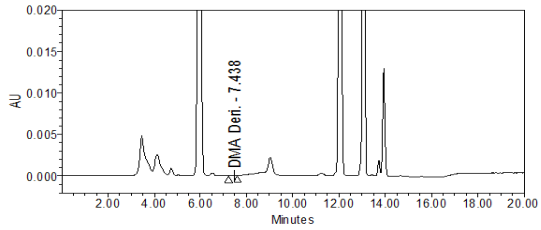

Fig. 5. Blank Chromatogram of HPLC derivatization method

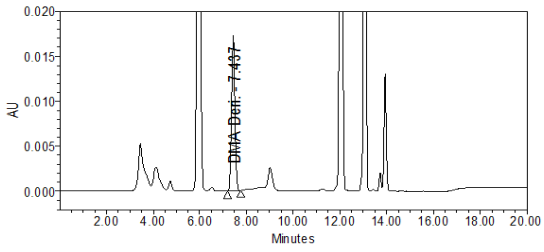

Fig. 6. Typical Chromatogram of DMA·HCl by HPLC derivatization method

The DMA content was determined using equation 2 given below.

$$DMA\ content = \frac{R_t - R_o}{R_s - R_o} \times \frac{C_s}{C_t} \times \frac{45.05}{81.55} \times P \qquad (2)$$

where R_t, R_s and R_o = detector response for DMA in sample solution, standard solution and blank solution respectively, C_s = concentration(in ppm) of DMA·HCl in standard solution, C_t = concentration (in ppm) of sample solution, P = Potency of dimethylamine hydrochloride, 45.05 and 81.55 are molecular weight of DMA and DMA·HCl, respectively.

3.2.5. Linearity

The linearity was evaluated and established by triplicate analysis of standard solution of DMA·HCl. The obtained peak areas were plotted against the corresponding concentration to generate calibration curve. (Fig. 7) Good linearity was evident (r^2 = 0.9999) over the examined

concentration range of 0.015 ppm to 0.75 ppm with equation y = 307998.47 x ± 266.34.

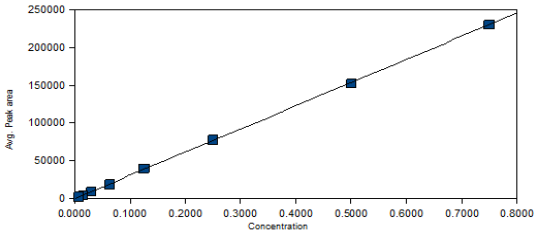

Fig. 7 Linearity plot of DMA·HCl by HPLC method

3.2.6. Method precision

The precision of the method, checked as the repeatability, was determined by calculating the relative standard deviation (% RSD) of the peak areas of DMA·HCl for six determinations made on the same day and under the same experimental condition. The % RSD of DMA·HCl peak area obtained by HPLC method is 0.99 % which is within limit not more than 10 %.

3.2.7. Accuracy and Recovery

According to ICH guidelines additional proof of accuracy was obtained by three replicate determinations of three different concentration solutions of DMA·HCl in IS solution in presence of MF·HCl API corresponding to 50 %, 100 % and 150 % of the analytical concentrations, respectively. Recovery studied by HPLC derivatization method, the obtained concentration values when compared to the nominal values produced accuracies of 100.67 %, 101.53 % and 100.12 % for the concentrations 50 %, 100 % and 150 % respectively , with an average % RSD value 0.71 %. The results of the method are within limit of accuracy 80 % to 120 % and % RSD not more than 10 %. Solution stability was checked for 42 hour by HPLC method and results were found to be very close to the result obtained with freshly prepared sample.

3.2.8. Comparison data of both techniques

The validation and performance characteristics of quantification of DMA·HCl obtained by ^{1}H qNMR were also compared with reversed phase derivatization HPLC techniques (Table 6) and the results of HPLC method are not much different from those of ^{1}H qNMR method.

3.2.9. Limitation with GC method

N,N-Dimethylamine (DMA) is a secondary amine. It is basic in nature with highly available non-bonding pair of electrons due to presence of dimethyl group, hence it interacts with the stationary phase of non-polar GC column which causes peak broadening, affecting the sensitivity. The conversion of DMA·HCl to DMA, which is volatile in nature, in presence of a base, for GC analysis can be considered but it degrades the active pharmaceutical ingredient MF·HCl which also generates DMA. So it is very difficult to quantify the DMA·HCl content in MF·HCl matrix by using GC technique as it shows much higher percentage of recovery than expected. Also, quantification by GC is laborious and time consuming which can be overcome by the proposed ^{1}H qNMR and derivatization HPLC techniques

Table 6. Performance characteristics of ^{1}H qNMR and HPLC method

Sr. No.	Study parameter	^{1}H qNMR	HPLC
1	Correlation coefficient (r^2)	0.9991	0.9999
2	Slope	0.7524	307998.47
3	Intercept	0.8303	-266.34
4	Limit of Detection (LOD)	4 ppm	0.037 ppm
5	Limit of Quantification (LOQ)	11 ppm	0.075 ppm

.3.2.10.Analysis of commercial sample

Samples from three different production batches (two batches, S-1 and S-2 form Sun Pharmaceutical Industries Limited, Vadodara, India, and one batch, W-1 from Wanbury Limited, Hyderabad, India) of MF·HCl were analyzed for the estimation of DMA·HCl by the proposed derivatization HPLC and ^{1}H qNMR methods and the observed results are given in Table 7.

Table 7. Commercial samples DMA·HCl content in MF·HCl by ^{1}H qNMR and HPLC.

Sample No.	Batch No.	^{1}H qNMR (ppm)	HPLC method (ppm)
1	S-1	72	65
2	S-2	24	19
3	W-1	60	52

The results show that both the methods are equivalent. Among the two, NMR method is simpler and quicker than HPLC method and can be easily adapted for the estimation of organic residual solvent DMA·HCl in MF·HCl for quality control and in-process analysis.

4. CONCLUSIONS

The ^{1}H qNMR method developed for the quantification of DMA·HCl in Metformin herein proved to be rapid as well as easy to perform. The method satisfies various performance criteria such as linearity, precision and accuracy. It offers an attractive choice over previously described

procedures and can be used for in-process, routine quality control for API and formulation analysis of Metformin hydrochloride tablets. Analysis can be carried out on any modern NMR equipment operating at a field of 400 MHz or more, equipped with suitable software for processing of data as per the protocol developed. Assay results obtained by ^{1}H qNMR have been confirmed by comparing them with a newly developed derivatization HPLC method, which has also been validated.

^{1}H qNMR method has a high potential in analysis of pharmaceutical products due to the simplicity, reliability, simultaneous identification and quantification, and the fact that there is no need of reference compound.

ACKNOWLEDGMENTS

The authors are thankful to Sun Pharmaceuticals Industries Limited, Tandalja, Vadodara, India, for providing all the facilities for this research work.

REFERENCES

[1] *British Pharmacopoeia*, Medicines and Healthcare products Regulatory Agency (MHRA). 5th Floor, 151, Buckingham Palace Road, London, SW1W9SZ.Volume-II (2012) 1414-1416.

[2] I.I. Hamdan, A.K. Jaber and A.M. Abushoffa, Development and validation of a stability indicating capillary electrophoresis method for the determination of metformin hydrochloride in tablets. *J. Pharm. Biomed. Anal.* 53 (2010) 1254-1257.

[3] C.J. Bailey, M.R.C. Path and R.C. Turner, Metformin, *N. Engl. J. Med*. 334 (2012) 574-579.

[4] A. Onal, Spectrophotometric and HPLC determinations of anti-diabetic drugs, rosiglitazone maleate and metformin hydrochloride, in pure form and in pharmaceutical preparations, *Eur. J. Med. Chem*. 44 (2009) 4998-5005.

[5] F. Al-Rimawi, Development and validation of an analytical method for metformin hydrochloride and its related compound (1-cyanoguanidine) in tablet formulations by HPLC-UV, *Talanta* 79 (2009) 1368-1371.

[6] K.M. Huttunen, J. Rautio, J. Leppänen, J. Vepsäläinen and P. Keski-Rahkonen, Determination of metformin and its prodrugs in human and rat blood by hydrophilic interaction liquid chromatography, *J. Pharm. Biomed. Anal*. 50 (2009) 469-474.

[7] A.R. Ali, I.I. Duraidi, M.M. Saket and E.S. Abu-Nameh, Column High-Performance Liquid Chromatographic Method for the Simultaneous Determination of Rosiglitazone and Metformin in a Pharmaceutical Preparation, *J. AOAC. Int*. 92 (2009) 119-124.

[8] D. Jain, S. Jain, D. Jain and M. Amin, Simultaneous Estimation of Metformin Hydrochloride, Pioglitazone Hydrochloride and Glimepiride by RP-HPLC in Tablet Formulation, *J. Chromatogr. Sci.* 46 (2008) 501-504.

[9] V. Porta, S.G. Schramm, E.K. Kano, E.E. Koono, Y.P. Armando, K. Fukuda and C.H. Serra, HPLC-UV determination of metformin in human plasma for application in pharmacokinetics and bioequivalence studies. *J. Pharm. Biomed. Anal*. 46 (2008) 143-147.

[10] J.Z. Song, H.F. Chen, S.J. Tian and Z.P. Sun, Determination of metformin in plasma by capillary electrophoresis using field-amplified sample stacking technique, *J. Chromatogr. B: Biomed. Appl.* 708 (1998) 277-283.

[11] I.H.I. Habib and M.S. Kamel, Near infra-red reflectance spectroscopic determination of metformin in tablets, *Talanta* 60 (2003) 185-190.

[12] M.A. Marques, Soares, S. Ade , O.W. Pinto, P.T. Barroso, D.P. Pinto, M. Ferreira-Filho and E. Werneck-Barroso, Simple and rapid method determination for metformin in human plasma using high performance liquid chromatography tandem mass spectrometry: Application to pharmacokinetic studies, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci*. 852 (2007) 308-316.

[13] H.N. Mistri, A.G. Jangid and P.S. Shrivastav, Liquid chromatography tandem mass spectrometry method for simultaneous determination of antibiotic drugs metformin and glyburide in human plasma, *J. Pharm. Biomed. Anal.* 45 (2007) 97-106.

[14] A.B. Van. Gysel and W. Musin, Methylamines Ullmann`s Encyclopedia of Industrial Chemistry, DOI: 10.1002/14356007(2005).a16-535.pub3.

[15] A.Q. Zhang, S.C. Mitschell and R.L. Smith, Dimethylamine formation in the rat from various related amine precursors, *Food. Chem. Toxicol.* 36 (1998) 923-927.

[16] International Conference on Harmonization, Harmonized Tripartite Guidelines, Validation of Analytical Procedures: Text and Methodology Q2 (R1) (2005).

[17] United States Pharmacopoeia and National Formulary (USP32/NF27, Rockville, Maryland, USA.United States Pharmacoepial Convention (2009).

[18] EDQM Public document. Content of the dossier for chemical purity and

microbiological quality. PA/PH/CEP (04) 14R, (2007).

[19] J.L. Jungnickel and J.W. Forbes, Quantitative Measurement of Hydrogen Types by Intergrated Nuclear Magnetic Resonance Intensities, *Anal. Chem.* 35 (1963) 938-942.

[20] D.P. Hollis, Quantitative Analysis of Aspirin, Phenacetin, and Caffeine Mixtures by Nuclear Magnetic Resonance Spectrometry, *Anal. Chem.* 35 (1963) 1682-1684.

[21] Z. Xia, L.G. Akim and D.S. Argyropoulos, Quantitative ^{13}C NMR Analysis of Lignins with Internal Standards, *J. Agric. Food. Chem.* 49 (2001) 3573-3578.

[22] U. Sahrbacher, A. Pehlke-Rimpf, G. Rohr, W. Eggert-Kruse and H.R. Kalbitzer, High resolution proton magnetic resonance spectroscopy of human cervical mucus, *J. Pharm. Biomed. Anal.* 28 (2002) 827-840.

[23] R.J. Wells, J.M. Hook, T.S. Al-Deen and D.B. Hibbert, Quantitative Nuclear Magnetic Resonance (qNMR) Spectroscopy for Assessing the Purity of Technical Grade Agrochemicals: 2,4-Dichlorophenoxyacetic Acid (2,4-D) and Sodium 2,2 Dichloropropionate (Dalapon Sodium), *J. Agric. Food. Chem.* 50 (2002) 3366-3374.

[24] P.A. Hays, Proton Nuclear Magnetic Resonance Spectroscopy (NMR) Methods for Determining the Purity of Reference Drug Standards and Illicit Forensic Drug Seizures, *J. Forensic. Sci.* 50 (2005) 1342-1360.

[25] V. Rizzo and V. Pinciroli, Qunatitative NMR in synthetic and combinatorial chemistry, *J. Pharm. Biomed. Anal.* 38 (2005) 851-857.

[26] A. Zoppi, M. Linares and M. Longhi, Quantitative analysis of enalapril by ^{1}H NMR spectroscopy in tablets, *J. Pharm. Biomed. Anal.* 37 (2005) 627-630.

[27] S.Y. Liu and C.Q. Hu, A comparative uncertainty study of the calibration of macrolide antibiotic reference standards using quantitative nuclear magnetic resonance and mass balance methods, *Anal. Chim. Acta.* 602 (2007) 114-121.

[28] G. Shao, R. Kautz, S. Peng, G. Cui and R.W. Giese, Calibration by NMR for quantitative analysis: *p*-Toluenesulfonic acid as a reference substance, *J. Chromatogr. A.* 1138 (2007) 305-308.

[29] E. Lopez-Rituerto, S. Cabredo, M. López, A. Avenoza, J.H. Busto and J.M. Peregrina, A Thorough Study on the Use of Quantitative ^{1}H NMR in Rioja Red Wine Fermentation Processes. *J. Agric. Food. Chem.* 57 (2009) 2112-2118.

[30] C.Y. Li, H.X. Xu, Q.B. Han and T.S. Wu, Quality assessment of Radix Codonopsis by quantitative nuclear magnetic resonance, *J. Chromatogr. A.* 1216 (2009) 2124-2129.

[31] P.A. Hays and R.A. Thompson, A processing method enabling the use of peak height for accurate and precise proton NMR quantitation. *Magn. Reson. Chem.* 47 (2009) 819-824.

[32] U. Holzgrabe, B.W. Diehl and I. Wawer, NMR spectroscopy in pharmacy, *J. Pharm. Biomed. Anal.* 17 (1998) 557-616.

[33] M. Imbenotte, N. Azaroual, B. Cartigny, G. Vermeersch and M. Lhermitte, Identification and quantitation of xenobiotics by ^{1}H NMR spectroscopy in poisoning cases, *Foren. Sci. Int.* 133 (2003) 132-135.

[34] A.A. Salem, H. A. Mossa and B.N. Barsoum, Quantitative determinations of levofloxacin and rifampicin in pharmaceutical and urine samples using nuclear magnetic resonance spectroscopy, *Spectrochim. Acta. Part A* 62 (2005) 466-475.

[35] A.A. Salem, H. A. Mossa and B.N. Barsoum, Applications of nuclear magnetic resonanace spectroscopy for quantitative analysis of miconazole, metronidazole and sulfamethoxazole in pharmaceutical and urine samples, *J. Pharm. Biomed. Anal.* 41 (2006) 654-661.

[36] L. Eirod, L.B. White and C.F. Wong, Determination of fortimicin a sulfate by high-performance liquid chromatography after derivatization with 2,4-dinitrofluorobenzene, *J. Chromatogr.* 208 (1981) 357-363.

[37] D.M. Barends, J.C.A.M Brouwers and A. Hulshoff, Fast pre-column derivatization of aminoglycosides with 1-fluoro-2,4-dinitrobenzene and its application to pharmaceutical analysis. *J. Pharm. Biomed. Anal.* 5 (1987) 613-617.

[38] Z. Chen, G. Xu, K. Specht and R. Yang, Determination of taurine in biological samples by reversed-phase liquid chromatography with precolumn derivatization with dinitrofluoro-benzene, *Anal. Chim. Acta.* 296 (1994) 249-253.

[39] L.T. Wong, A.R. Beaubian and A.P. Pakuts, An HPLC radiotracer method for assessing the ability of L-Cysteine prodrugs to maintain glutathione levels in the cultured rat lens, *Curr. Eye. Res.* 15 (1996) 501-510.

[40] U. Holzgrabe, B. Diehl and I. Wawer, *NMR Spectroscopy in Pharmaceutical Analysis*. Elsevier Ltd., Oxford, UK (2008) 49-50.

[41] M.M. Malet and U. Holzgrabe, NMR techniques in biomedical and pharmaceutical analysis, *J. Pharm. Biomed. Anal.* 55 (2011) 1-15.