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Quantitative determination and Validation of Pioglitazone in Pharmaceutical using Quantitative Nuclear Magnetic Resonance Spectroscopy

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ABSTRACT

Rapid, specific and accurate proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) method was developed to determine Pioglitazone hydrochloride antidiabetic drug in pharmaceutical tablet formulation. The method was based on quantitative NMR spectroscopy (qNMR) using Maleic acid as an internal standard and deuterated dimethylsulfoxide (DMSO-d6) as an NMR solvent. For the quantification of the drug, the ^{1}H-NMR signals at 7.95 ppm and 6.26 ppm corresponding to the analyte proton of Pioglitazone drug and Maleic acid internal reference standard (IS) respectively were used. The method was validated for the parameters of specificity and selectivity, precision and intermediate precision, linearity, limit of detection (LOD) and limit of quantification (LOQ), range, accuracy, solution stability and robustness. The linearity of the calibration curve for analyte in the desired concentration range is good (R^2 =0.9983). The method was accurate and precise with good recoveries. Range study was also performed up to saturation level (226.93 mg/0.60 mL) in DMSO-d6. The advantage of the method is that no reference standard of analyte drug is required for quantification. The method is nondestructive and can be applied for quantification of Pioglitazone hydrochloride in commercial formulation products.

Keywords: Pioglitazone hydrochloride, qNMR, Quantitative NMR spectroscopy, Validation, ^{1}H NMR spectroscopy.

INTRODUCTION

Nuclear magnetic resonance (NMR) spectroscopy is a quantitative spectroscopic tool because the intensity of a resonance line is directly proportionate to the number of resonant nuclei. 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. The high selectivity under appropriate acquisition conditions and the possibility of performing quantitative analysis without analyte standards are the most attractive features of this technique. Quantitative

determination is normally obtained from the ratio between the integration of a specific signal of the analyte and the internal reference standard (IS).

Quantitative measurement was first described in 1963 by Jungnickel and Forbes [1] and Hollis [2]. Despite limited accuracy, quantitative ^{1}H-NMR find application in various fields of science [3-13]. The lack of absorbing chromophores for UV(ultraviolet)–visible detection and the need for the special chromatographic detectors as well as the difficulties in establishing highly efficient solid or liquid phase extraction procedures have made NMR most suitable for biological sample analysis of many drugs[14-17].

Pioglitazone hydrochloride, (±)-5-{4-[2-(5-ethyl-2-pyridyl) ethoxy] benzyl}-2,4-thiazolidinedione hydrochloride salt (Fig. 1a), is an oral antidiabetic agent that has been shown to affect abnormal glucose and lipid metabolism associated with insulin resistance by enhancing insulin action on peripheral tissues in animals. It is used in the treatment of type-II diabetes.

Several analytical methods have been reported for the determination of Pioglitazone hydrochloride in bulk drug form, pharmaceuticals and biological fluids. These mainly include chromatographic techniques in association with UV [18-20], MS [21-22] (Mass spectroscopy), TLC [23-24] (Thin layer chromatography) and MEKC [25] (Miceller electrokinetic chromatography) etc. As per literature search, there are many analytical methods available for the quantification of drug but almost all the methods are based on lengthy chromatographic techniques. To the best of our knowledge, no official method has been reported by qNMR. Hence the present study has been undertaken. The aim of this work is to develop advantageous and competitive selective NMR method for the determination of the drug in formulation as well as in API samples that complies well with the validation requirements in the pharmaceutical industry.

EXPERIMENTAL SECTION

Reagents and Standards

High purity analytical grade substances were used throughout. Authentic sample of Pioglitazone hydrochloride was obtained from local pharmaceutical company. Maleic acid (99.90%) and deuterated dimethylsulfoxide (DMSO-d6) (99.99%) were purchased from Merck. PIOGLIT 30 - Extended Release Tablets containing 30 mg Pioglitazone were purchased from local market [Manufactured by Sun Pharmaceutical Ltd., India].

Instrumentation

NMR: Bruker AV300 FT-NMR spectrometer operating at a frequency 300.13 MHz (7.1 Tesla) for protons, equipped with a 5 mm ^{1}H-^{13}C dual probehead and 5 mm multinuclear observe (BBO) probehead.

Preparation of standard and test solutions

Standard Preparation

Accurately weighed pure Pioglitazone hydrochloride (equivalent to 10.00 mg Pioglitazone drug) was thoroughly mixed with Maleic acid (5.00 mg) IS. The mixture was dissolved in 0.6 ml of DMSO-*d*6. Solution was thoroughly mixed till complete dissolution.

Pioglitazone Standard Preparation for Specificity
Accurately weighed pure Pioglitazone hydrochloride (equivalent to 10.00 mg Pioglitazone drug) and transferred to stoppered tube and 0.6 ml of DMSO-d6 was added. Solution was thoroughly mixed till complete dissolution.

Maleic Acid IS Preparation for Specificity
Accurately weighed and transferred 5.00 mg of Maleic acid to stoppered tube and 0.6 ml of DMSO-d6 was added. Solution was thoroughly mixed till complete dissolution.

Placebo Solution Preparation for Specificity
Accurately weighed and transferred 47.00 mg of placebo (mixture of excipients without drug) to stoppered tube and 0.6 mL of DMSO-d6 was added. Solution was thoroughly mixed till complete dissolution and supernatant was taken for analysis.

Standard Preparation for Robustness study (IS Variation: 5.0 ±1.0 mg)
Accurately weighed and transferred pure Pioglitazone hydrochloride (equivalent to 10.00 mg Pioglitazone drug) into two different stoppered tubes and added 4.20 mg and 6.10 mg of Maleic acid IS to both stoppered tube respectively. Then 0.6 ml of DMSO-d_6 was added. Solution was thoroughly mixed till complete dissolution.

Sample Preparation (Tablets)
Ten tablets of PIOGLIT-30 were weighed, crushed and thoroughly ground into fine powder. Portion equivalent to 10.00 mg Pioglitazone was weighed accurately and transferred to stoppered tube. An appropriate amount of accurately weighed Maleic acid (5.00 mg) and 0.6 ml of DMSO-d6 were added. Solution was thoroughly mixed till complete dissolution and supernatant was taken.

Sample Preparation for Robustness study (IS Variation: 5.0 ± 1.0 mg)
Portions equivalent to 10.00 mg Pioglitazone was weighed from ground tablet sample and transferred into two different stoppered tubes and added 4.17 mg and 6.15 mg of Maleic acid IS to stoppered tube respectively. Then 0.6 ml of DMSO-d_6 was added. Solution was thoroughly mixed till complete dissolution.

NMR analysis
^{1}H NMR spectra of authentic drug and tablet samples were measured using 300 MHz, BRUKER-AV300 spectrometer. 64 scans were collected for each sample into 32,768 data points using a 30^o pulse length; spectral width 6172.839 Hz; digital resolution 0.188380 Hz/points; preaquisition delay 6 μs and acquisition time 2.654 s. A delay time of 11 s between pulses was used to ensure fully T_1 relaxation of protons.

The FIDs were apodized with 0.3 Hz exponential line broadening function before fourier transformation. Manual two-parameter phase correction was used to obtain high quality absorption line shape followed by baseline correction. This manual mode was also used for the signal integration. Chemical shifts were referenced internally to residual dimethylsulfoxide signal obtained at δ = 2.49 ppm.

Procedure for ^{1}H-NMR method

Performed standard preparation in replicate (n=6) and sample preparation in triplicate. Recorded ^{1}H-NMR under the experimental conditions given as per NMR analysis section. Integrated analyte ^{1}H signal obtained at 7.95 ppm with respect to ^{1}H signal of maleic acid IS at 6.26 ppm.

Calculations [26]

The amount Wx and assay Px of drug was calculated using the following equations:

$$W_x = \frac{I_x}{I_{Std}} \frac{N_{Std}}{N_x} \frac{M_x}{M_{Std}} m_{Std} \quad (1)$$

$$P_x = \frac{I_x}{I_{Std}} \frac{N_{Std}}{N_x} \frac{M_x}{M_{Std}} \frac{m_{Std}}{m} P_{Std} \quad (2)$$

Where,
***W**x = Weight of Pioglitazone drug (in mg)*
***P**x = Assay of the Pioglitazone (in %w/w) on as is basis*
***I**x = Mean Integral value of the analyte ^{1}H signal (doublet) obtained at 7.95 ppm*
***I**std = Integral value of the ^{1}H signal of Maleic acid IS obtained at 6.26 ppm*
***N**std = Number of protons for the Maleic acid IS*
***N**x = Number of protons for the analyte ^{1}H in drug*
***M**x = Molar mass of the analyte drug (For Pioglitazone 356.45 gm/mole)*
***M**std = Molar mass of the Maleic acid IS (116.07 gm/mole)*
***m**std = Weight of the Maleic acid IS. (in mg)*
***m** = Taken weight of the analyte drug (in mg)*
***P**std = Assay of the maleic acid IS (99.90%)*

RESULTS AND DISCUSSION

NMR Experiments for confirmation of Structure Characterization

Fig. 1 shows the structure of analyte drug and Maleic acid IS with its assignments. ^{1}H-NMR, Deuterium exchange (D_2O-X) NMR, ^{13}C NMR, Distortionless Enhancement by Polarization Transfer (DEPT), 2-D ^{1}H-^{1}H correlation spectroscopy (COSY) and 2-D ^{1}H-^{13}C heteronuclear single quantum correlation (HSQC) NMR experiments were performed for confirmation of structure characterization of Pioglitazone HCl drug. It was helpful during assignment of ^{1}H signals of the drug. The ^{1}H-NMR of Maleic acid IS was also done in DMSO-d6 solvent for confirmation of its structure.

Determination of relaxation time T_1

For accurate quantification, proper value of relaxation delay is very important. The relaxation delay (t) depends on the longest longitudinal relaxation time T_1 of all signals of interest. The T_1 relaxation is described by

$$M_Z = M_0\left(1 - e^{-(t/T_1)}\right) \quad (3)$$

with Mz and M_0 being the magnetization along the z-axis after waiting time t and at thermal equilibrium, respectively. A delay of five T_1's is normally sufficient between the last RF pulse and the application of the next RF pulse [27].

The relaxation time T_1 was determined experimentally by inversion recovery experiment for all the protons of the drug and internal reference standard (Table 1). The longest relaxation time 2.128 s was found for the Maleic acid IS and for the proton of interest of drug was 0.74 s. Therefore, 11 s delay time between pulses was enough to ensure fully T_1 relaxation of protons.

Assignment of ^{1}H NMR signals of the drug and IS

Fig. 2 and fig. 3 show the ^{1}H NMR and ^{13}C NMR spectrum of Pioglitazone hydrochloride. The 3 protons of $–CH_3$ of ethyl group, assigned as *a*, attached to 2-pyridine ring gave triplet at 1.18-1.23 ppm and 2 protons of $–CH_2$ of same ethyl group, assigned as *b*, showed quartet at 2.73-2.81 ppm. The 2 methylene groups assigned as *f* and *g* gave triplet at 3.47-3.51 ppm and 4.36- 4.40 ppm respectively. The 2 protons assigned as *j* and *k* of methylene group gave 2 signals of double doublets at 3.02-3.07 ppm and 3.24-3.30 ppm respectively. Methine proton assigned as *l* showed multiplet at 4.83-4.87 ppm. Doublets observed at 6.84-6.87 ppm and 7.11-7.14 ppm were due to aromatic benzene protons assigned as *h,h'* and *i,i'* respectively. Protons of 2-pyridine ring assigned as *d* and *c* were observed as singlet and doublet at 8.71 ppm and 8.39-8.41 ppm respectively. The isolated broad singlet appeared at 12.05 ppm due to imide proton (-NH) assigned as *m* was also confirmed by deuterium exchange NMR experiment. The isolated, doublet signal appeared at 7.95-7.98 ppm can be attributed to the proton of 2-pyridine ring assigned as *e* was selected for quantitative determination of the drug. The isolated, sharp singlet signal at 6.26 ppm was due to methylene protons of Maleic acid IS assigned as IS_H and was used as standard signal for quantitative determination (Fig. 4). The other signals obtained at 2.49 ppm and 3.30 ppm are due to residual solvent and water of solvent of the DMSO-d6 respectively.

Additionally, assignment of protons of drug molecule had been confirmed with D_2O exchange, DEPT, 2-D ^{1}H-^{1}H COSY and 2-D ^{1}H-^{13}C HSQC experiments (Data was not shown). Fig. 1 and Table 1 summarize band assignments for all signals.

Quantitative NMR method

The signal intensity of a known amount of an IS was compared to the area of the peaks originating from the analyte. In the current study, the IS chosen was Maleic acid, since it supplies a well-separated signal without any interference from analyte drug signal in the integration region. Of all the common internal standards used in our lab, this was the best choice with respect to both solubility and the chemical shifts of the different protons compared to the drug and other substances in the samples. The singlet of Maleic acid chosen for quantification was assigned a value of 2.00 in each NMR spectra.

For Pioglitazone hydrochloride, the doublet at 7.95 ppm, originating from one proton of the 2-pyridine ring was used, since this peak appears well separated from other signals. The ^{1}H NMR spectrum of standard and sample preparation in DMSO-d6 shows a well-separated doublet of analyte proton and the singlet of the Maleic acid IS. (Fig. 5)

Validation of method

The method was validated as per International Conference on Harmonization (ICH) guidelines[28] for following parameters- system suitability, specificity and selectivity, precision and intermediate precision, linearity, LOD and LOQ, range, accuracy and robustness.

System suitability

System suitability – to show that the control measures required have been complied with for a particular analysis on a particular day, a system suitability check is required. Such a check on the performance of the spectrometer and method may be used, for example, to ensure that the expected specificity and sensitivity can be achieved. One of the advantages of the use of NMR as a quantitation method is that the sample itself may provide such system suitability test by, for example, making use of line-width or S/N data in the sample spectrum. Because of the high precision and intrinsic accuracy, system precision for NMR is not required. How ever system precision was performed for every parameter by replicate acquisitions of standard preparation. It was called as system suitability test and checked the compliance of acceptance criteria as mentioned below.

% Relative standard deviation (RSD) of the integral value of analyte signal should not be more than 2.00 [29], Signal to Noise Ratio (S/N) of the analyte signal should be more than 150 [26, 29] and Difference of the δppm value of analyte signal should not be more than 0.2 ppm. All above three acceptance criteria are defined in-house. Because in quantification, it is already accepted that first two parameters means peak area (integration) and S/N ratio of analyte signal are very critical for accurate and precise results. Moreover, another important parameter-chemical shift is also included here because the position of analyte peak should be identified properly.

A result of the system suitability was meeting the acceptance criteria at each validation study. Means system was precise and suitable for analysis.

Specificity and selectivity

The selectivity and specificity of proposed method was evaluated through possible interference due to the presence of the excipients in the pharmaceutical formulations.

Specificity study was performed by analyzing the diluent (DMSO-d6), placebo solution preparation, Pioglitazone standard preparation, Maleic acid IS preparation and sample (tablet) preparation. It was concluded that there was no interference at the signals obtained at 7.95 ppm and 6.26 ppm for analyte proton & IS respectively due to diluent & placebo. Also the signals of the analyte proton and Maleic acid IS were well separated from each other in standard and sample preparations (Fig. 6).

Precision & Intermediate Precision

The precision of an analytical method expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample. The precision of the integration procedure of qNMR depends on the Signal to Noise ratio (S/N) of the signals of interest. S/N of at least 150:1 is necessary for every resonance line; which should be integrated, for a precision better than 99% or an uncertainity of 1% [26]. Also according to the ICH guidelines the precision will be acquired by six repeated determinations (n=6) and

intermediate precision will be evaluated by a second analyst and/or a second NMR spectrometer with a different magnetic field strength.

The precision was assessed by 6 separate sample preparations. Calculated the content of drug in mg and %assay for each preparation and statistical results were tabulated (Table 2). S/N ratio for each measurement was calculated and found to be more than 150:1.

The biggest factor of influence on the quality of a qNMR analysis is the handling of the NMR data by different operators [26]. Integration of peaks as well as phase and baseline correction is the most subjective parts of the method. Therefore, intermediate precision was determined by performing measurements on three different occasions. Six different sample preparations were prepared and analyzed on 5 mm multinuclear BBO probehead by different analyst and on different day. The average of six analyses, standard deviation and relative standard deviation values are documented in Table 2. Precision and intermediate precision results did not show any marked differences.

Linearity

qNMR as a method itself is linear because the intensity of the response signal is directly proportional to the amount of nuclei contributing to this signal.

Linearity was checked by preparing standard solutions at seven different concentration levels ranging from 75% to140%, according to the content of analyte in test sample. Linearity curve was drawn for taken drug amount (in mg) vs. found drug amount (in mg). The equation for curve was y = 1.0201x - 0.1307. The correlation coefficient was found 0.9983, indicating good linearity. (Fig. 7)

LOD & LOQ

In the case of NMR with lorentzian lines as response signals, the LOD and LOQ have to be calculated by the standard deviation of the response σ and the slope S of a calibration curve obtained in Linearity study. The LOD and LOQ were calculated using Eq. (4) and Eq. (5) respectively [30]**.**

$$\mathrm{LOD} = \frac{3.3\sigma}{\mathrm{S}} \quad (4)$$

$$LOQ = \frac{10\sigma}{S} \quad (5)$$

LOD and LOQ were found to be 0.48 mg and 1.48 mg per ml of diluent respectively.

Range

The range study was determined by preparing solutions of drug up to saturated concentration in solution. Saturated solution was prepared by adding excess drug amount and analyzing supernatant solution for determining the dissolved concentration of drug. Saturation concentration was found 226.93 mg per 0.60 ml diluent.

Accuracy

The accuracy of an analytical method expresses the closeness of agreement between an accepted reference value and the value found. The accuracy of an analytical procedure should be established across its range. The ICH documents recommend that accuracy should be assessed using a minimum of nine determinations over a minimum of three concentration levels, covering the specified range (i.e. three concentrations and three replicates of each concentration).

Data from nine determinations over three concentration levels covering the specified range was determined. The accuracy was studied at 80%, 100% and 120% levels with respect to the sample by preparing the solutions in triplicate at each level. From the results as per Table 3, it was concluded that method for assay content was accurate between the ranges of 80% to 120% level. %RSD at each level was found to be less than 2.00.

Stability of analyte in solution

Stability of analytes (and standard) over the analysis period – self-evidently the system under test must not change during the test if the results from the test are going to be meaningfully related to the original sample. The solution is said stable, if % difference in assay is not more than 1.0 when compared to initial value. If solution is not stable at room temperature, same study is repeated at refrigeration temperature (~2-8°C).

Standard preparation and sample preparation were analyzed at ambient temperature (~25^{o}C) at 0hr (Initial), 6hrs, 12hrs, 18hrs & 24hrs intervals and calculated %assay for each interval. Measured %difference for both preparations at different time intervals with respect to the corresponding initial value and found no major change. Results are tabulated in Table 4.

Robustness

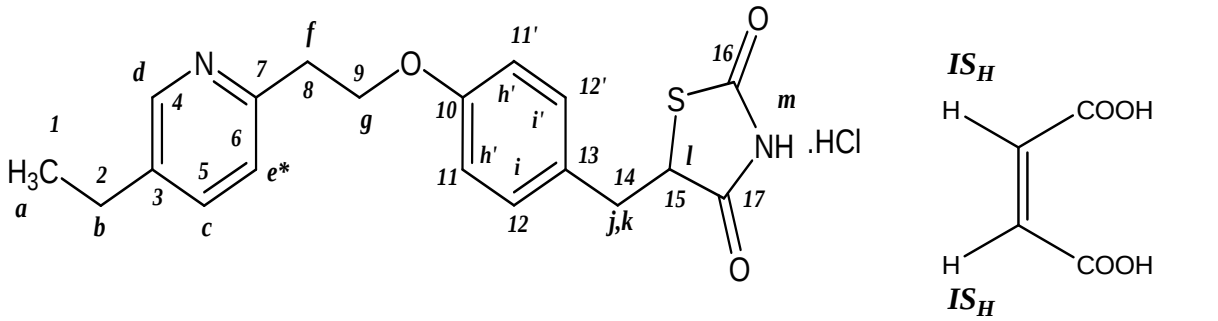
The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in procedural parameters listed in the procedure documentation and provide an indication of its suitability during normal usage.

The robustness of the method was evaluated by varying three parameters independently: (1) The Internal standard amount (5mg±1) (2) The number of scans (64scans±16) and (3) Choosing different analyte proton (at 8.7 ppm). All samples were prepared fresh daily.

A variation of 20% in internal standard amount did not appreciably change the measured amount of drug. Running the experiment using a different number of scans such as 48 or 80 rather than 64 also did not affect the measurement. Selection of different analyte proton obtained at 8.7 ppm also gave similar results. Thereby, this method is quite robust in terms of the above-mentioned parameters (Table 5).

Comparison with other technique (HPLC)

Assay results obtained by qNMR were also confirmed by comparing with other in-house HPLC technique. It was found that results of HPLC method did not show any marked differences with qNMR method.



(a) **(b)**

Fig. 1 Structure of (a) Pioglitazone HCl with ^{1}H & ^{13}C assignment and (b) Maleic acid IS with ^{1}H assignment

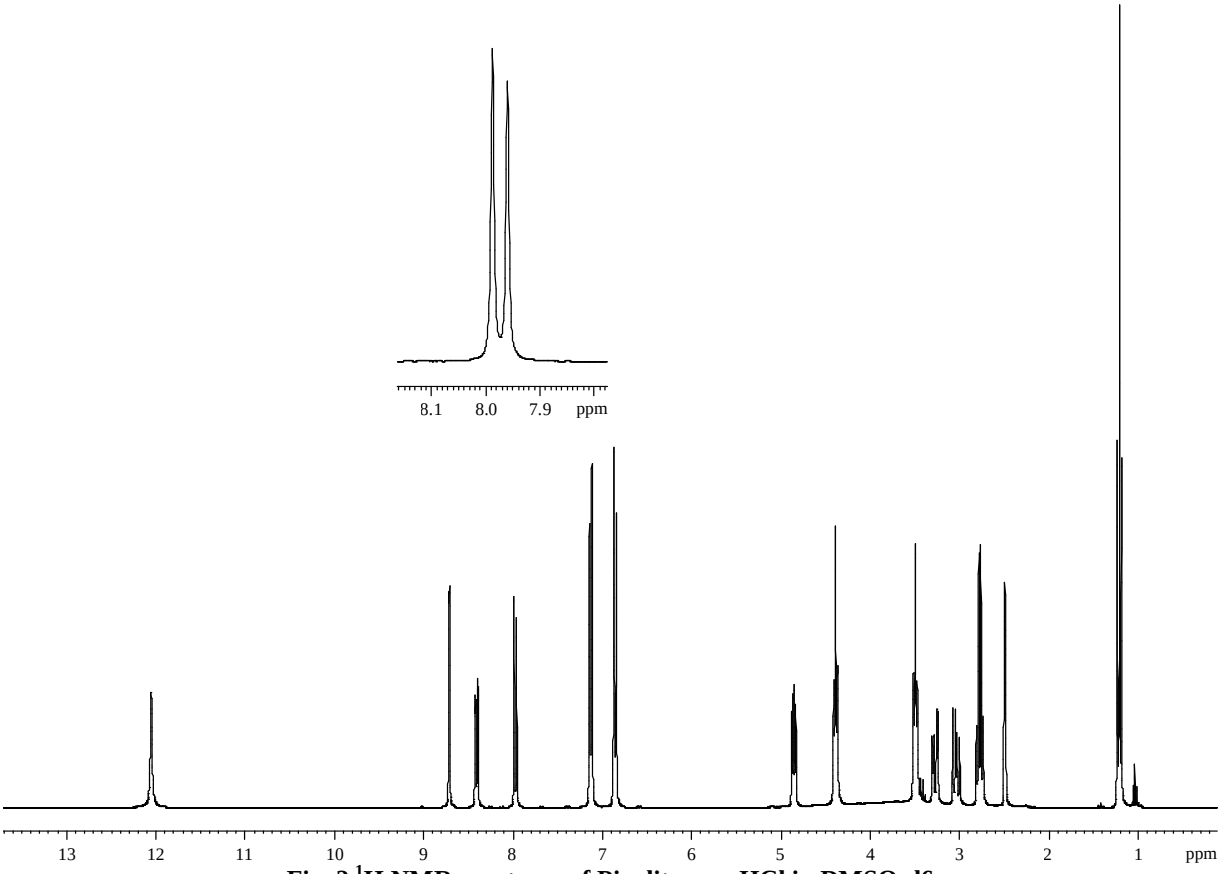


Fig. 2 ^{1}H NMR spectrum of Pioglitazone HCl in DMSO-d6

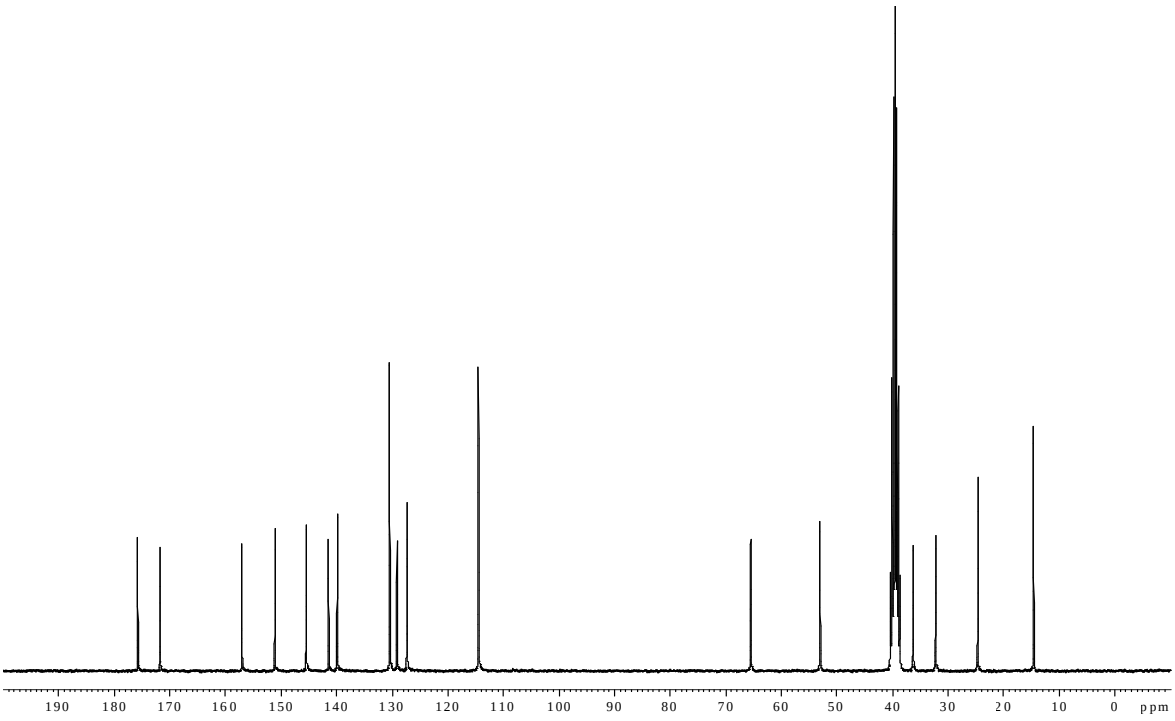


Fig. 3 ^{13}C NMR spectrum of Pioglitazone HCl in DMSO-d6

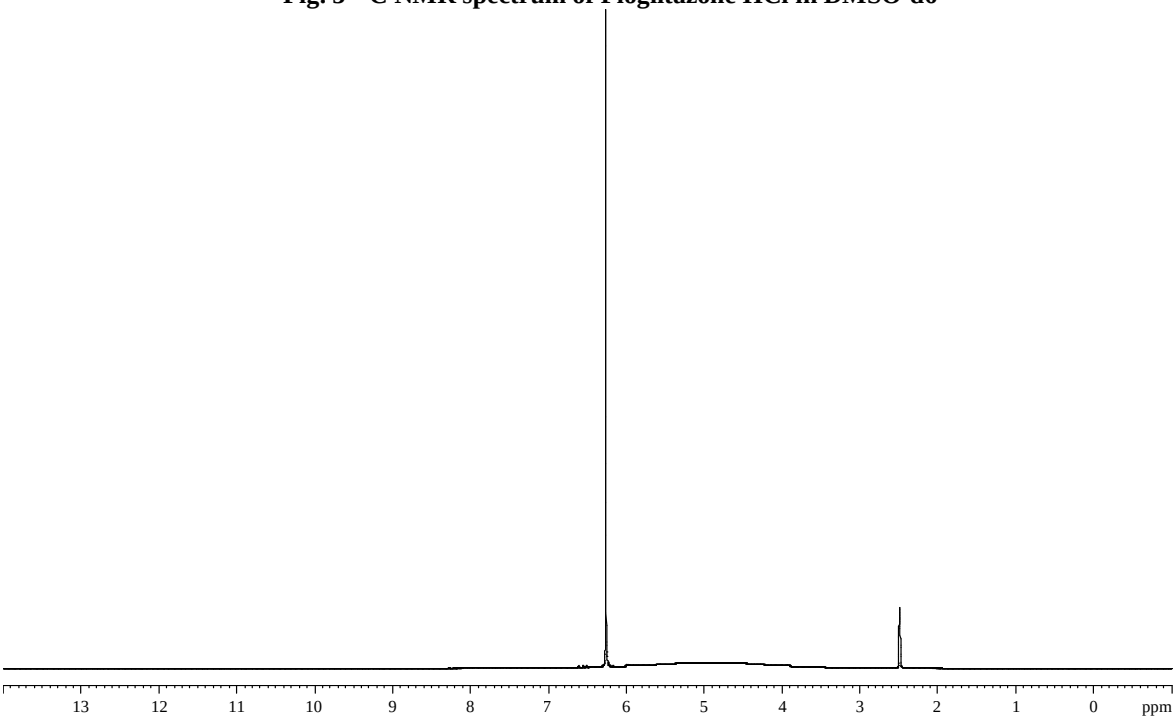


Fig. 4 ^{1}H NMR spectrum of Maleic Acid IS in DMSO-d6

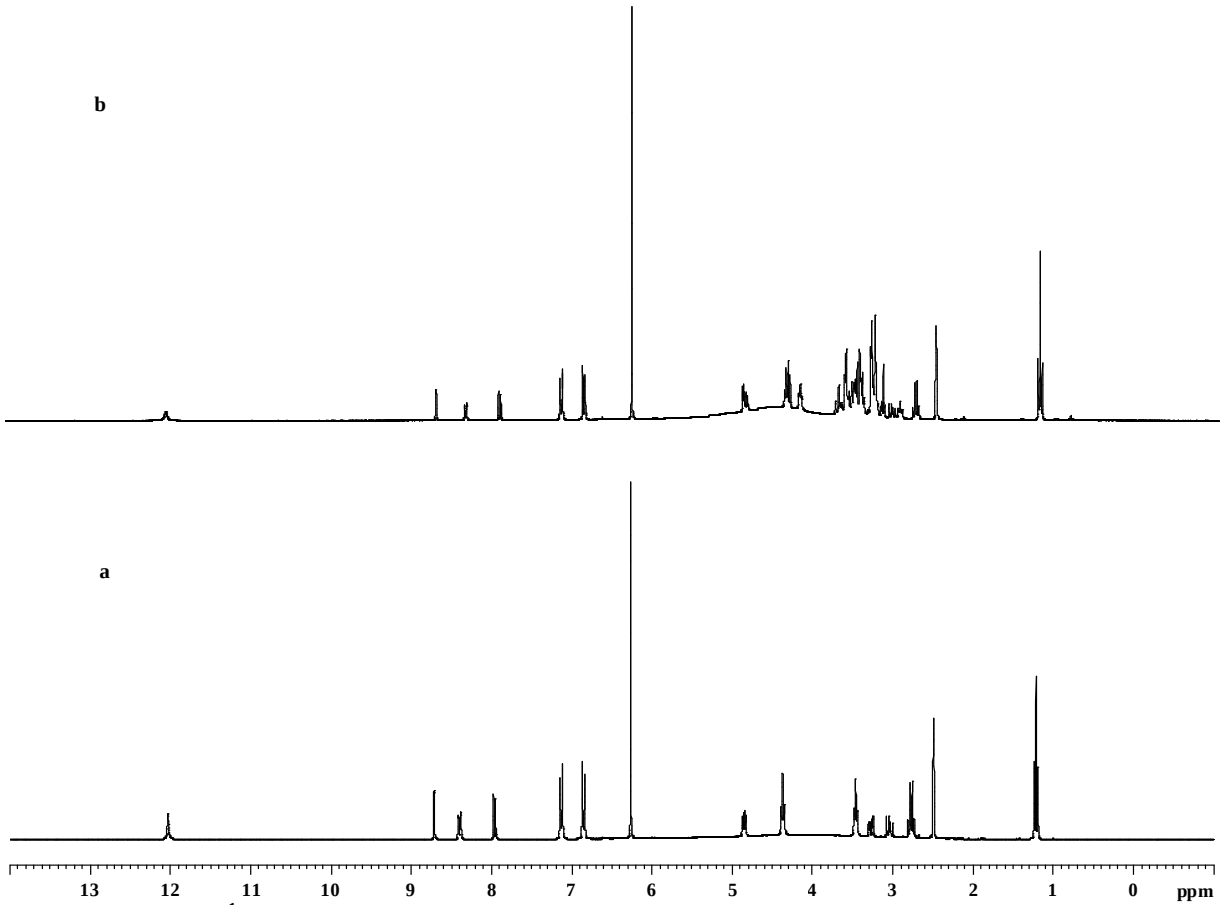


Fig. 5 ^{1}H NMR spectrum of (a) Standard preparation and (b) Sample preparation

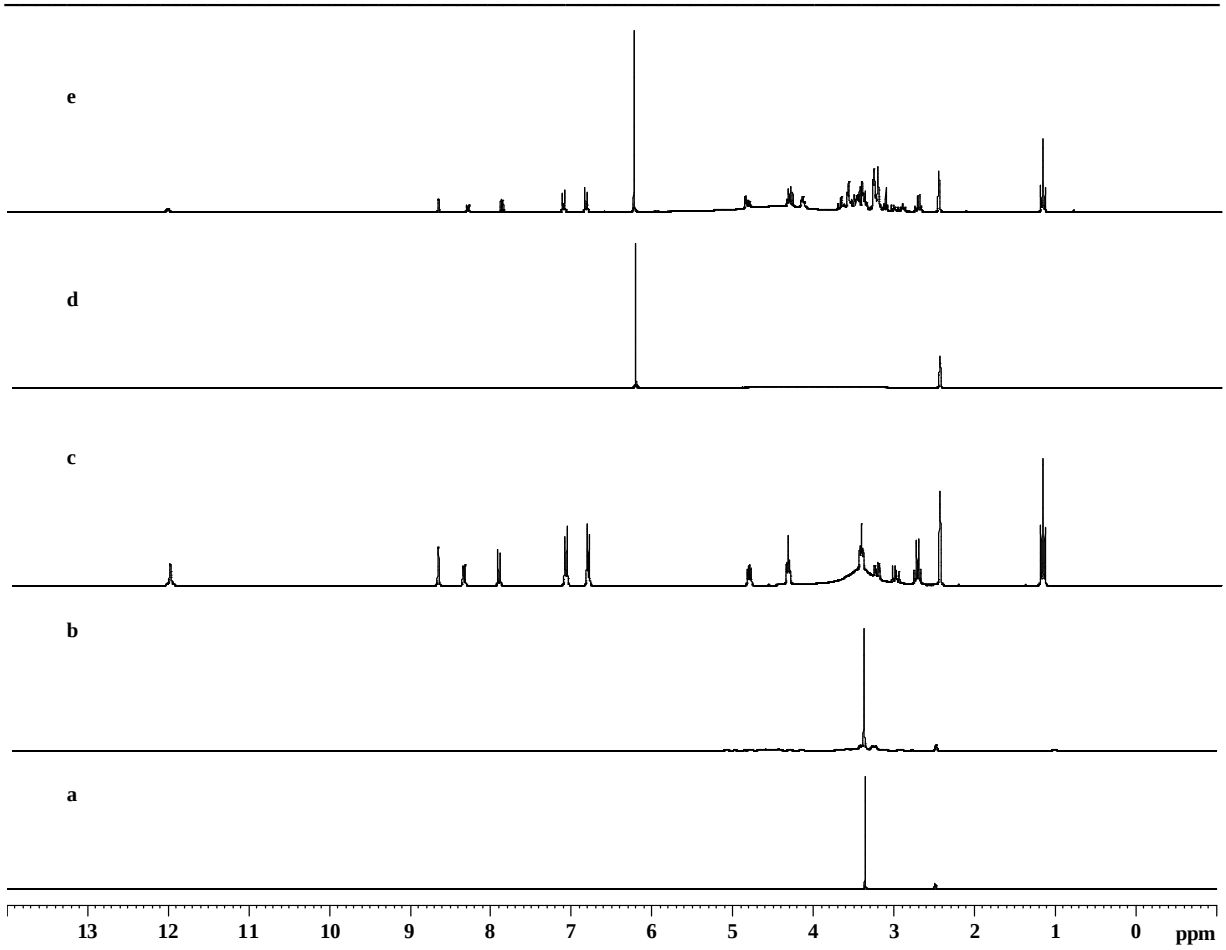


Fig. 6 ^{1}H NMR spectra of (a) DMSO-d6 diluent, (b) Placebo, (c) Pioglitazone HCl, (d) Maleic Acid IS and (e) Sample preparation

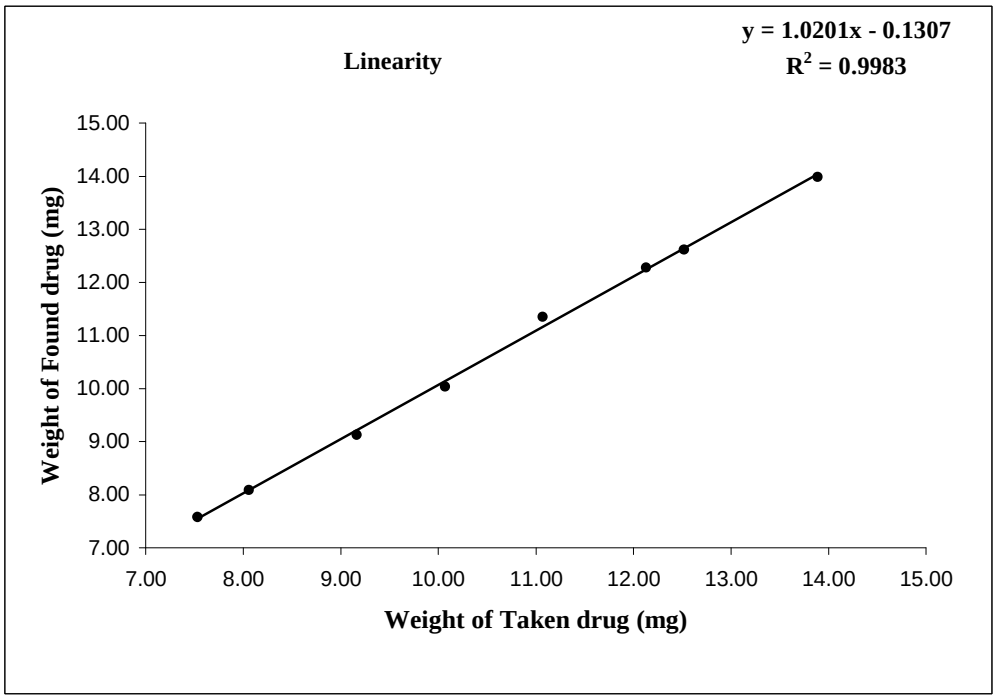


Fig. 7 Linearity curve of found drug amount in mg vs. taken drug amount in mg

Table 1 ^{1}H NMR &^{13}C NMR assignments with T_1 for Pioglitazone HCl and Maleic acid IS

Pioglitazone HCl drug							
	^{1}H NMR				**^{13}C NMR**		
Type of Protons	**Assign as [#]**	**^{1}H-δppm with multiplicity**	**T_1 (sec)**	**Type of Carbons**	**Assign as [#]**	**^{13}C-δppm**	**DEPT**
3H (methyl-CH_3)	*a*	1.18-1.23(t)	0.999	1-CH_3 (Aliphatic)	*1*	14.062	1 x -CH_3
2H (methylene-CH_2)	*b*	2.73-2.81(q)	0.567	1-CH_2 (Aliphatic)	*2*	24.643	1 x -CH_2
1H (2-pyridine)	*c*	8.39-8.41(d)	0.871	1-C (2-pyridine)	*3*	151.084	-
1H (2-pyridine)	*d*	8.71(s)	0.744	1-CH (2-pyridine)	*4*	139.862	1 x -CH
1H (2-pyridine)	*e**	7.95-7.98(d)	0.740	1-CH (2-pyridine)	*5*	145.437	1 x -CH
2H (methylene-CH_2)	*f*	3.47-3.51(t)	0.255	1-CH (2-pyridine)	*6*	127.303	1 x -CH
2H (methylene-CH_2)	*g*	4.36-4.40(t)	0.252	1-C (2-pyridine)	*7*	157.034	-
2H (2-CH Benzene)	*h,h'*	6.84-6.87(d)	0.752	1-CH_2 (Aliphatic)	*8*	32.185	1 x -CH_2
2H (2-CH-Benzene)	*i,i'*	7.11-7.14(d)	0.812	1-CH_2 (Aliphatic)	*9*	65.490	1 x -CH_2
1H (methylene-CH_2)	*j*	3.02-3.07(dd)	0.275	1-C (1-benzene)	*10*	141.450	-
1H (methylene-CH_2)	*k*	3.24-3.30(dd)	0.274	2-CH (1-benzene)	*11,11'*	114.463	2 x -CH
1H (methine-CH)	*l*	4.83-4.87(m)	0.872	2-CH (1-benzene)	*12,12'*	130.456	2 x -CH
N-H (imide)	*m*	12.05(bs)	0.628	1-C (1-benzene)	*13*	129.092	-
Maleic Acid IS				1-CH_2 (Aliphatic)	*14*	36.261	1 x -CH_2
2H-(ethylene)	IS_H	6.22(s)	2.128	1-CH (Aliphatic)	*15*	52.997	1 x -CH
				1-C (1-amide)	*16*	171.712	-
				1-C (1-amide)	*17*	175.724	-

** signal selected for quantification; [#] Refer structure (Fig. 1) for assignment;*
s=singlet, d=doublet, t=triplet, dd=double doublet, brs=broad singlet, m=multiplet

Table 2 Precision and Intermediate Precision test results

Study		Precision			Intermediate precision	
Preparation	Taken drug (mg)	*Found drug (mg)	%Assay	Taken drug (mg)	*Found drug (mg)	%Assay
1	10.12	9.93	98.04	10.02	9.77	97.40
2	10.10	10.03	99.20	10.05	9.89	98.29
3	10.06	9.89	98.22	10.03	9.80	97.61
4	10.08	9.91	98.19	10.09	9.93	98.39
5	10.02	9.89	98.67	10.12	9.92	97.91
6	10.03	9.79	97.46	10.04	9.83	97.75
		Mean	98.30		Mean	97.89
		SD	0.5894		SD	0.3854
		%RSD	0.60		%RSD	0.39

** average of three determinations.*

Table 3 Accuracy Test Results

Accuracy level	Taken drug (mg)		*Found drug (mg)	%Assay
80% Set-1	8.07		7.96	98.57
80% Set-2	8.11		7.92	97.66
80% Set-3	8.05		7.88	97.76
100% Set-1	10.02		9.85	98.22
100% Set-2	10.05		9.86	97.98
100% Set-3	10.03		9.88	98.36
120% Set-1	12.04		11.73	97.31
120% Set-2	12.02		11.77	97.80
120% Set-3	12.12		11.91	98.16
			Mean	97.98
		Overall	SD	0.3881
			%RSD	0.40

** average of three determinations.*

Table 4 Stability of analyte in solution test results

	For Standard Preparation				For Sample Preparation			
Time Interval (Hours)	Taken drug (mg)	*Found drug (mg)	% Assay	% Difference	Taken drug (mg)	*Found drug (mg)	% Assay	% Difference
Initial	10.61	10.57	99.53	NA	10.06	9.89	98.15	NA
After 6	10.61	10.55	99.26	0.27	10.06	9.87	98.02	0.13
After 12	10.61	10.52	98.99	0.54	10.06	9.84	97.68	0.48
After 18	10.61	10.54	99.21	0.32	10.06	9.85	97.80	0.35
After 24	10.61	10.52	98.97	0.56	10.06	9.87	97.97	0.18

** average of three determinations.*

Table 5 Results for Robustness study

Parameter	Change	*Found drug (mg)	%Assay	%Difference
Number of Scan	48	9.81	97.86	0.25
	64	9.92	98.11	NA
	80	9.85	98.22	0.11
Internal Std (mg)	4.17	9.95	98.31	0.20
	5.40	9.92	98.11	NA
	6.15	9.89	98.19	0.08
Analyte Proton	7.9 ppm	9.92	98.11	NA
	8.7 ppm	9.90	97.99	0.12

** average of three determinations.*

CONCLUSION

The qNMR method employed herein proved to be rapid as well as easy to implement. The different aspects of performance of the method, such as linearity, precision and accuracy, satisfied our requirements well. It offers an excellent choice over previously described procedures and can be used for routine quality control and stability analysis of Pioglitazone hydrochloride in solid dosage forms. Assay results obtained by qNMR were confirmed by comparing with in-house HPLC method. Furthermore, any modern NMR equipment operating at a field of 300MHz or more may be used, assuming that suitable processing of data is performed.

qNMR has a high potential in analysis of pharmaceutical products due to the simplicity, reliability, simultaneous identification and quantification, and the fact that no reference compound of drug is needed.

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REFERENCES

[1] JL Jungnickel; JW Forbes. *Anal Chem.,* **1963**, 35, 938–942.
[2] DP Hollis. *Anal Chem*., **1963**, 35, 1682–1684.
[3] SY Liu; CQ Hu. *Anal Chim Acta*., **2007**, 602, 114-121.
[4] G Shao; R Kautz; S Peng; G Cui; RW Giese. *J Chromatogr A*., **2007**, 1138, 305-308.
[5] V Rizzo; V Pinciroli. *J Pharm Biomed Anal*., **2005**, 38, 851-857.
[6] A Zoppi; M Linares; M Longhi. *J Pharm Biomed Anal*., **2005**, 37, 627-630.
[7] PA Hays. *J Forensic Sci*., **2005**, 50, 1342-1360.
[8] PA Hays; RA Thompson. *Magn Reson Chem*., **2009**, 47, 819- 824.
[9] U Sahrbacher; A Pehlke-Rimpf; G Rohr; W Eggert-Kruse; HR Kalbitzer. *J Pharmaceut Biomed.,* **2002**, 28, 827–840.
[10] RJ Wells; JM Hook; TS Al-Deen; DB Hibbert. *J Agric Food Chem*., **2002**, 50, 3366–3374.
[11] Z Xia; LG Akim; DS Argyropoulos. *J Agric Food Chem*., **2001**, 49, 3573–3578.

[12] E López-Rituerto; S Cabredo; M López; A Avenoza; JH Busto; JM Peregrina. *J Agric Food Chem.*, **2009**, 57, 2112-2118.
[13] CY Li; HX Xu; QB Han; TS Wu. *J Chromatogr A.*, **2009**, 1216, 2124-2129.
[14] U Holzgrabe; BWK Diehl; I Wawer. *J Pharm Biomed Anal*., **1998**, 17, 557–616.
[15] M Imbenotte; N Azaroual; B Cartigny; G Vermeersch; M Lhermitte. Foren Sci Int., **2003**, 133, 132–135.
[16] AA Salem; HA Mossa; BN Barsoum. *Spectrochim Acta Part* A., **2005**, 62, 466–472.
[17] AA Salem; HA Mossa; BN Barsoum. *J Pharm Biomed Anal*., **2006**, 41, 654-661.
[18] E Tahmasebi; Y Yamini; A Saleh. *J Chromatogr B Analyt Technol Biomed Life Sci*., **2009**, 877, 1923-1929.
[19] C Giaginis; S Theocharis; A Tsantili-Kakoulidou. J Chromatogr B., **2007**, 857, 181-187.
[20] P Sripalakit; P Neamhom; A Saraphanchotiwitthaya. *J Chromatogr* B., **2006**, 843, 164-169.
[21] M Wang; IR Miksa. *J Chromatogr B.*, **2007**, 856, 318-327.
[22] ZJ Lin; W Ji; D Desai-Krieger; L Shum. *J Pharm Biomed Anal*., **2003**, 33, 101-108.
[23] A Gumieniczek; H Hopkala; A Berecka. *J Liq Chromatogr Relat Technol*., **2004**, 27, 2057-2070.
[24] RTS Menon; S Inamdar; M Mote; A Menezes. *J Planar Chromatogr Mod TLC*., **2004**, 17, 154-156.
[25] T Radhakrishna; D Sreenivas; GO Reddy. *J Pharm Biomed Anal*., **2002**, 29, 593-607.
[26] F Malz; H Jancke. *J Pharm Biomed Anal*., **2005**, 38, 813-823.
[27] U Holzgrabe; B Diehl; I Wawer. NMR spectroscopy in pharmaceutical analysis. **2008**, pp 49
[28] ICH Harmonized Tripartite Guidelines. Validation of Analytical Procedures: Text and Methodology Q2 (R1). **2005**.
[29] G Maniara; K Rajamoorthi; R Srinivasan; GW Stockton. *Anal Chem*., **1998,** 70, 4921-4928.
[30] MIRM Santero; NM Kassab; AK Singh; E Kedor-Hackmam. *J Pharm Biomed Anal*., **2006,** 40, 179-184.