

Recent Development in Nuclear Magnetic Resonance as a Technique for Quantitative Method Validation

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Sahu *et al.*: Review on Use of Nuclear Magnetic Resonance for Quantitative Method Validation

Nuclear magnetic resonance spectroscopy has largely given way for most important routine analysis of natural, synthetic drug candidate and related molecules. The quality control and simultaneous quantitation of different residual solvents, analytes and impurities have led analysts to further exploit nuclear magnetic resonance as a viable quantitative technique. Hence, this review focuses on the use and feasibility of this technique for quantitative method validation of drug and related compounds. Validated quantitative nuclear magnetic resonance methods were simple, robust, accurate and selective for evaluating different natural and synthetic samples using various commercially accessible compounds as internal standards. In this review numerous examples are cited giving proof of advancement in nuclear magnetic resonance technology and also for precise evaluation of molecular structures of substances in solids and liquid. Studied examples of different concurrent validation methods in the review shows that the results obtained is precise with less standard deviation when compared with other chromatography techniques. Concluding the entire review, various methodologies used for quantification and validations were reflected that proved to be a specific, selective and alternative means of method validation and it can be summarized that nuclear magnetic resonance is as reliable as the chromatography-based technique for quantitative analysis providing accuracy.

Key words: Quantitative nuclear magnetic resonance, validation, internal standard, relative standard deviation, solvent

Nuclear Magnetic Resonance (NMR) spectroscopy is an illustrious analytical technique for structure elucidation of simple as well as complex molecules and applied in various branches of bio-sciences, chemistry, physics and medical diagnosis. Purcell and Bloch in 1945, discovered the phenomenon of Proton Nuclear Magnetic Resonance (¹H NMR) in solids and liquids^[1-6], and its application in quantitative analysis was reported in the year of 1963 by Forbes and Jungnickel^[7]. NMR has been considered as a rapid, convenient, reliable and non-destructive method for quantitative evaluations and is a crucial analytical tool for the structural determination of unknown synthetic as well as natural compounds^[8-13]. Additionally, it has the inherent benefits of giving simultaneous access to both qualitative and quantitative evaluation; latter is defined by the foremost ratio rule: the signal intensity is directly proportional to the number of nuclei that give rise due to a specific resonance. Advancement in NMR instrumentation and technology for instance high field

magnets, solvent suppression techniques, cryoprobes and versatile pulse sequences has been leading many analysts to further exploit NMR as viable quantitative techniques. Nowadays, quantitative NMR (qNMR) measurements are reliable and precise as those results obtained by the more commonly used chromatography-based techniques. NMR technique provides advantages like: simple development of method, ease of sample preparation, relatively less analysis times and multiple calibration possibility without the need for similar reference materials^[14-23].

In the well-recognized magnetic resonance technique, for the determination of nuclei magnetic moments

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by energy transitions, molecular beams are induced between levels of energy which coincide with different orientations of the nuclear spin in applied constant, strong strength of magnetic fields. Experiments are accomplished in a consistent magnetic field B_0 (various Tesla) aligned along with the z-axis. As an outcome of this field, the space is no more isotropic and nuclei spins interaction will depend on the alignment of the molecule with due regard to the magnetic field B_0 ^[24-32].

The foremost claims of one dimensional (1D) quantitative ^1H NMR (qH-NMR) are the purity evaluation of organic compounds and the determination of possible potential impurities and development of second frequency dimension in NMR spectroscopy leads to direct association with the identification of molar concentration in a provided sample. In one set of two-dimensional NMR (2D-NMR) experiments, the Hamiltonian is swapped between the evolution period and the detection period. As a result of the evolution period, resonances are expanding into a second dimension to disclose their origins^[19,33-43].

High-resolution NMR methods have been implemented for spin quantum $I=1/2$ (^1H , ^{19}F , ^{31}P , etc.)^[44], ultrafast Magic Angle Spinning (MAS) up to 70 kHz at ultra-high field (23.3 T) and efficient homonuclear decoupling in the case of strongly coupled nuclei. In parallel, ingenious intense resolution approach have been implemented for quadrupolar nuclei ($I>1/2$: ^{17}O , ^{23}Na , ^{27}Al , etc.)^[45], for instance Dynamic-Angle Spinning (DAS), Double Rotation (DOR), Multiple-Quantum Magic-Angle Spinning (MQ-MAS) and Satellite-Transition Magic-Angle Spinning (ST-MAS). All of these approaches enable the analyst to determine the asymmetric unit of a desired structure and the isotropic distribution of chemical shifts and quadrupolar factors^[46-51].

The NMR signals intensities (is equal to areas under specific signals) can be taken for quantitative evaluations as the signal intensity (I) is directly correlated to the number of nuclei (N) bringing the signal. The linear association between the intensity of signal (I) and the number of possible nuclei ((N) in case of single pulse excitation) is given by:

$$[I=C_s \times N]$$

The proportionality constant (C_s) results from different parameters of the spectrometer termed as “spectrometer constant”. Further the validation of qNMR methods and the accuracy of the integrals measuring the correctness of quantitation depend on factors like; (a) the spectrum

noise level (S/N ratio), (b) shimming quality, (c) the shape, (d) baseline, drift and phase corrections and (e) choice of the window functions^[52,53].

Perhaps the main restraint of qNMR technique is the need of human intervention throughout processing operations that nearly impact integral values (e.g. the integrated signals choice, integral tails setting and phasing). The quantitative accuracy deviation in qNMR has been reported to be $<2.0\%$, which is an acceptable limit for accurate and precise quantification^[54-56]. NMR spectroscopy is a well-recognized analytical technique for qualitative and quantitative method validation (e.g., precision, linearity, accuracy, robustness, reproducibility, specificity and selectivity)^[57]. Unlike other approaches, qNMR spectroscopy has certain processing and acquisition parameters and referencing approaches that needs careful consideration in order to acquire certain intense degree of precision and accuracy. One must take care of sample preparation and experimental approaches as they may stimulate significant errors in quantitative determination, thereby reduces the accuracy and the precision of the observing data^[58].

Solvents used in NMR and solvent locking concept:

In both ^1H and Carbon-13 (^{13}C) NMR techniques Tetramethylsilane (TMS) or related compounds, are used as an internal reference which gives proton signal at 0.0 ppm and the carbon signal at 0.0 ppm in the NMR spectrum. However, in current scenario of advancements in NMR spectroscopy “solvent lock” concept has been taken into consideration. By using this concept addition of internal reference in solvent is not required as spectrometers can “lock” on solvent signals. The ^1H and ^{13}C NMR chemical shifts have been compiled for common laboratory solvents often used for analysis of compounds or contaminants in samples (examples of various selected solvents are given in Table 1 and Table 2)^[59-63].

QUANTITATIVE METHOD VALIDATION OF DRUGS AND EXCIPIENTS USING NMR TECHNIQUE

NMR spectrometer is a quantitative spectroscopic device, because the intensity of a peak or resonance line is directly corresponding to the number of resonant nuclei (spins). This fact permits, for a precise determination of the quantity of compounds in liquids as well as solids. Due to use of stronger and high steady magnetic fields including enhanced electronics the distinguishing

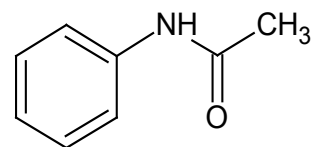
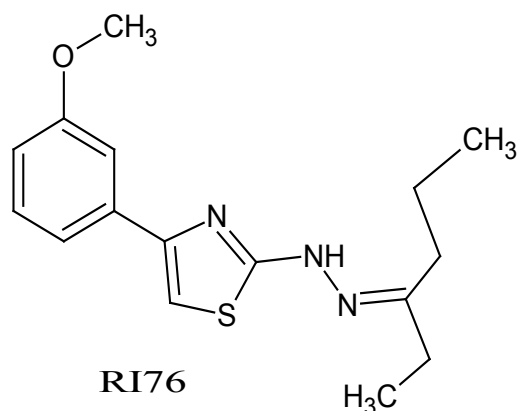
limits have been poked down remarkably. Sometimes due to contemplation of accuracy protocol, control of evaluation procedure aspects, spectral processing and assessment which are responsible for the quantitative investigations of similar samples in diverse laboratories may differ severely (deviations in up to 90 % relative to gravimetric reference values). It considers all issues regarding linearity, specificity, robustness, selectivity and accuracy as well as influences of instrument specific parameters and the data processing and evaluation routines^[64-66].

TABLE 1: ¹H NMR CHEMICAL SHIFT DATA

Solvent (Deuterated)	Solvent residual peak (ppm)	H ₂ O/Deuterated water (HOD)
Deuterated chloroform (CDCl ₃)	7.26	
Acetone ((CD ₃) ₂ CO)	2.05	1.56
Dimethyl sulfoxide ((CD ₃) ₂ SO)	2.50	2.84
Benzene (C ₆ D ₆)	7.16	3.33
Acetonitrile (CD ₃ CN)	1.94	0.40
Methanol (CD ₃ OD)	3.31	2.13
2,2,2-Trifluoroethanol (TFE-d ₃)	5.02, 3.88	4.87
	4.79	3.66
Deuterium oxide (D ₂ O)		

TABLE 2: ¹³C NMR CHEMICAL SHIFT DATA

Solvent (Deuterated)	Solvent signal (ppm)
CDCl ₃	77.16±0.06
Acetone ((CD ₃) ₂ CO)	29.84±0.01
CD ₃ ₂ SO	39.52±0.06
Benzene (C ₆ D ₆)	128.06±0.02
Acetonitrile (CD ₃ CN)	1.32±0.02
Methanol (CD ₃ OD)	49.00±0.01
2,2,2-Trifluoroethanol (TFE-d ₃)	61.50, 126.28
Deuterium oxide (D ₂ O)	-

**Fig. 1: Chemical structure of the compound RI76 and acetanilide^[67]**

Quantitative validation of a novel anti-fungal drug (RI76):

Validation of RI76 (a novel anti-fungal drug) was accomplished on a Bruker 400 MHz Avance spectrometer by utilizing both the internal standard (IS) and the Electronic Reference to Access *In Vivo* Concentrations (ERETIC-2) calibration methods. The parameters for the quantitative evaluation were as follows: 16 scans, single 30° pulse, acquisition time of 4.12 s, relaxation delay of 15 s, a Spectrum Width (SW) of 20 ppm with the transmitter offset set at 7 ppm.

Acetanilide (99 %), Fumaric acids United States Pharmacopeia (USP) (100 %) were utilized as IS and as a reference for the ERETIC-2 calibration, respectively. In the experiment, 5 mg of acetanilide and 12 mg of RI76 (fig. 1) were accurately weighed and dissolved in 600 µl of Deuterated dimethyl sulfoxide (DMSO-d₆), which was accurately transferred to NMR tubes for evaluating the purity of RI76^[67].

For the quantitation study, the RI76 signal at 7.76 ppm (doublet, 2H) and the acetanilide reference signal at 2.04 ppm (singlet, 3H) were considered. Purities ranging from 60 to 70 % and relative standard deviation (RSD) 1.91 % were observed. The remaining 30 % of the mass comprises impurities. The study revealed that equivalent results were observed with both IS and ERETIC-2 calibration approaches yield the same result^[67].

Quantitative validation of alpha (α) bisabolol:

Validation of α-bisabolol was done by using Varian Mercury 300 MHz and Bruker Avance III 400 MHz spectrometer for quantitation in essential oils of *Eremanthus erythropappus* which was suitable for most of the samples evaluated (E1, E2, E3, E4, E5, E7, E10 and E11).

A stock solution of α -bisabolol (105.0 mg/ml) and octamethylcyclotetrasiloxane (OMCTS; 1.9 mg/ml) were prepared to measure linearity, accuracy, precision and stabilities of α -bisabolol and OMCTS in solution (fig. 2).

Taken essential oil comprise of small quantities of other constituents hence the signal at 5.13 and 5.36 ppm of α -bisabolol is not overlapped with signals from the other constituents present in the essential oil. Through the $^1\text{H-NMR}$ spectral signals at 5.36 and 5.13 ppm, the Limit Of Detection (LOD) and Limit Of Quantification (LOQ) were observed as 0.26 and 2.59 mg, respectively. The study revealed that purity of α -bisabolol was 95.5 % and RSD or coefficient of variation (CV) was <1.75 %. The method for quantitative validation of α -bisabolol was found rapid, efficient and appropriate^[68].

Quantitative validation of α -pinene:

α -Pinene is a natural terpene isolated from *Corymbia*, *Eucalyptus* and turpentine oils and its validation was accomplished on Varian Mercury 300 MHz spectrometer which was equipped with a 5 mm $^1\text{H-}^{13}\text{C}$ dual probe head. The acquisition parameters for the quantitative measurement considered were 16 scans, pulse breadth 45° (pb45), 25 temperature of the samples in the spectrometer, 5 min acquisition time and automatically shimming was carried out.

A stock solution of α -pinene (98.0 mg/ml) and OMCTS; 2.2 mg/ml) were prepared in Deuterated chloroform (CDCl_3) to determine linearity, robustness and stability of the NMR method (fig. 3). An exact amount (from 22.0 to 46.0 mg) of each essential oil sample was weighed directly into the NMR tube in which 310 μl of the OMCTS stock solution was added.

The ^1H NMR spectra were observed and the signal of OMCTS at 0.10 ppm was integrated. The signals at

5.17-5.23 ppm were integrated in all samples and the values of the integrations were used to calculate the amount of α -pinene which lies in between 98-102 %. The LOD and LOQ were 0.1 and 2.5 mg, respectively and RSD or CV was observed <0.76 %.

The quantitation method developed by using ^1H NMR was adequate for α -pinene quantification from *Corymbia* and *Eucalyptus* since various sources of essential oils were successfully measured. The study revealed that method used for quantification of α -pinene was efficient and precise^[69].

Quantitative validation of cellulose in tobacco:

Bruker Avance III 400 MHz spectrometer was used in the study for efficient and rapid quantitative validation of cellulose in tobacco which was equipped with 7 mm $^1\text{H-}^{13}\text{C}$ double resonance MAS probe head. Commercially tobacco samples with inconsistent cellulose content were collected from Guizhou, China.

Evaluation of cellulose in tobacco was done by employing ^{13}C cross polarization/MAS (^{13}C CP/MAS) with following parameters: the initial proton/2 pulses was 5 s, the 90° pulse length, contact time was 2 min for all tobacco sample, the recycle delay was 2 s and the acquisition time was 25 min. Sample cells were cylindrical zirconium-dioxide rotors of 7 mm diameter with silicon tube inserts as an IS and cell spun at 4 kHz. The weight of each tobacco sample was noted during sample loading and ranged from 100 to 300 mg.

^{13}C CP/MAS NMR spectra of tobacco samples were processed with spectral deconvolution to acquire the area of the C-1 resonance at 105.5 ppm and the IS at 0 ppm. The spectra of untreated tobacco sample and standard cellulose are compared.

The results unfolded that the average value for five measurements was 8.62 % with the absolute value

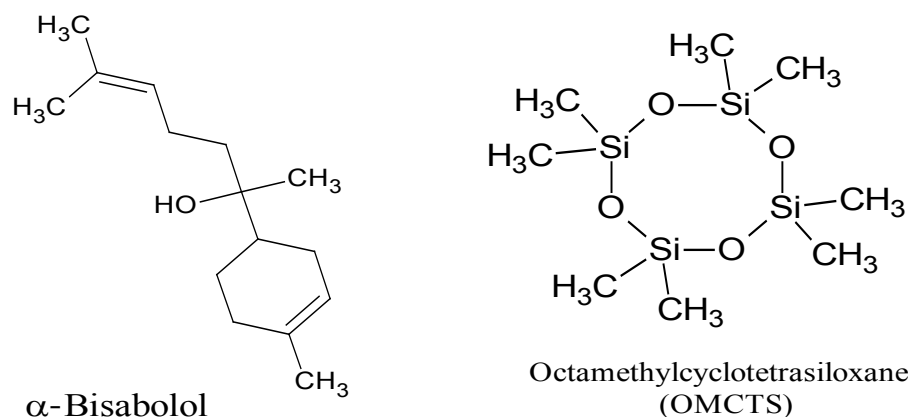
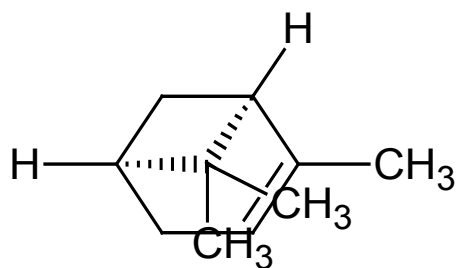
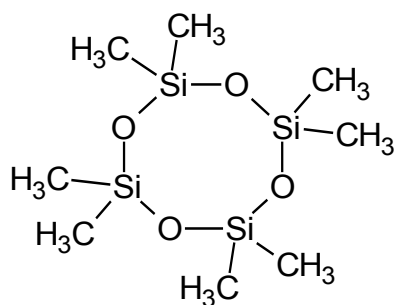


Fig. 2: Chemical structure of the studied compound α -bisabolol and OMCTS^[68]

 α -PineneOctamethylcyclotetrasiloxane
(OMCTS)**Fig. 3: Chemical structure of α -pinene and OMCTS^[69]**

of the RSD was <1.49 %. These data suggest that the proposed method can be used for quantitative analysis of cellulose in tobacco with a quite high degree of consistency among the results under the same conduction^[70].

Quantitative validation of amygdalin:

Validation of amygdalin content in Persicae semen, Armeniacae semen and Mume fructus were accomplished on JEOL JMN-ECA-500 MHz spectrometer with the following parameters for each sample: 8 scans were recorded with pulse angle 90° and 60 s pulse delay time which was greater than the spin-lattice relaxation time (>5×T₁).

A stock solution of hexamethyldisilane (HMD) (10.0 mg) in pyridine-d₅ (10.0 ml) was prepared for quantitative determination of amygdalin (fig. 4) and its concentration was determined using quantitative ¹H NMR with reference bisphenol-A (10.0 mg in 1 ml stock solution).

The amygdalin purity was precisely calculated from the ratio of the intensity of the amygdalin H-2 signal at 6.50 ppm in pyridine-d₅ to that of the HMD signal at 0 ppm is shown. The study revealed the amygdalin contents to be 2.72 and 3.13 % in 2 lots of Persicae semen, 0.23 % in Mume fructus and 3.62 and 5.19 % in 2 lots of Armeniacae semen^[71].

Quantitative validation of a generic natural product (Ginkgolides):

Validation of complex natural products (Ginkgolide-B and Ginkgolide-A) were completed on Bruker Avance III 400 MHz spectrometer which was equipped with a 5 mm TXI cryoprobe with the following parameters: spin-lattice relaxation times (T₁) were measured by an inversion recovery experiment (T₁-IR; 12 increments

from 50 min to 60 s) for Dimethyl Sulfone (DMSO₂) (2.480 s), Dimethyl Sulfoxide (DMSO-d₅) (12.658 s) and caffeine (doublet H 8.000 ppm: 2.973 s; doublet H 3.868 ppm: 1.756 s) is shown here.

The caffeine and DMSO₂ were used as calibrants for External Calibration (EC) and the residual solvent signal DMSO-d₅ was used for Internal Calibration (IC). Samples were prepared by mixing and diluting stock solutions (cDMSO₂=24.5 mg/ml; cCaffeine=27.5 mg/ml) with six different sample concentrations were separately weighed (concentration range 3.3-40.4 mM) into the NMR tubes and dissolved in 0.6 ml DMSO-d₅. The DMSO-d₅ concentration was evaluated using the calibration curve for y=1 as 65.0 mM (0.54 %).

The spectra represent the highest S/N for the respective window function: (A) Caffeine spectra (H-8) were used in DMSO-d₅ (600 MHz), applying exponential multiplication (EM, line broadening factor (LB)=3), Gaussian multiplication (GM, LB=3) and Lorentzian Gaussian multiplication (LG, LB=0.3, GF=0.05) in NMR Utility Transform Software (NUTS). (B) The DMSO-d₅ residual solvent signal (J=1.7 Hz) under the same processing conditions as (A).

The spectral region having the H-12 was used for quantitative evaluation of the four terpene lactones exist in the mixture. The ECIC method yielded data that revealed the absolute measurement of Ginkgolide-B as 2.87 mM in a 5.66 mM sample by weight, revealing a purity of only 51.3 %. The data as well allowed for the identification of 0.45 % Ginkgolide-A purity at a S/N of about 13 in the 2.87 mM sample of Ginkgolide-B^[72].

Quantitative validation of medicinal plant extracts and herbal products:

Validation of extracts from medicinal plants *Eugenia jambolana* (*E. jambolana*), *Aegle marmelos*

(*A. marmelos*) and *Withania somnifera* (*W. somnifera*) and their herbal products (as mentioned in fig. 5) were done using Bruker Avance III 400 MHz spectrometer which was equipped with a 5 mm multinuclear inverse probe head with the following parameters: pre-acquisition delay of 6 min, acquisition time of 4.0 s, recycle delay of 5.0 s and a flip angle of 30°.

1,3,5-Trimethoxybenzene (TMB 1.94, 3.23 mg) was utilized as an IS for evaluation of *E. jambolana* and *A. marmelos*, while 2,4-diformyl phloroglucinol (1.42 mg) was utilized for *W. somnifera*. Both these standards are freely soluble in CDCl₃ solvents. TMB had shown two singlets in the spectra, the methoxy

proton resonance at d 3.7 and aromatic protons at d 6.09 while 2,4-diformyl phloroglucinol had shown two singlets, for the formyl proton at d 10.05 and for aromatic proton at d 5.89. The reference signals at 9.11, 9.04 and 9.07 for H-4 protons of malvidin, delphinidin and petunidin diglucosides with integrals of 0.37, 0.22 and 0.27, respectively, were utilized for analysis of anthocyanin with the IS, TMB having an integral of 10.0 for three protons of aromatic.

The crude extracts of *W. somnifera* (IS 2,4-diformyl phloroglucinol) had shown a distinct signal for olefinic proton (H-2) at d 6.19 and for the formyl protons at d 10.05 of 2,4-diformylphloroglucinol. The proton

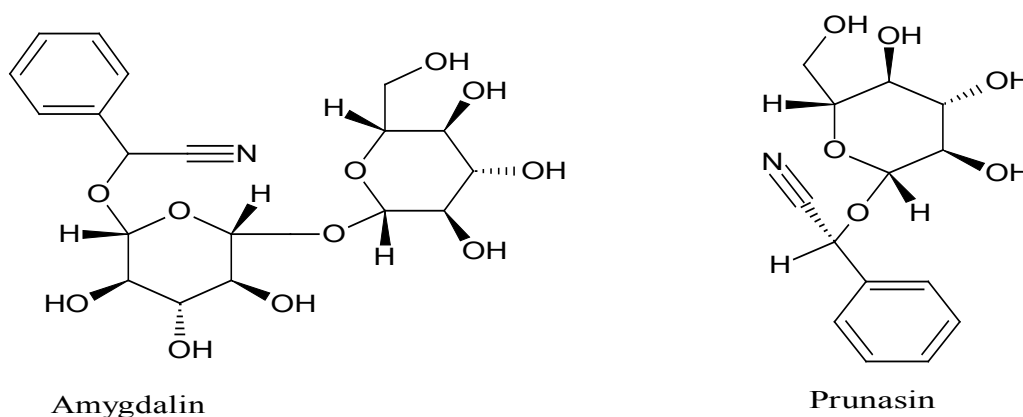


Fig. 4: Chemical structure of amygdalin and prunasin^[71]

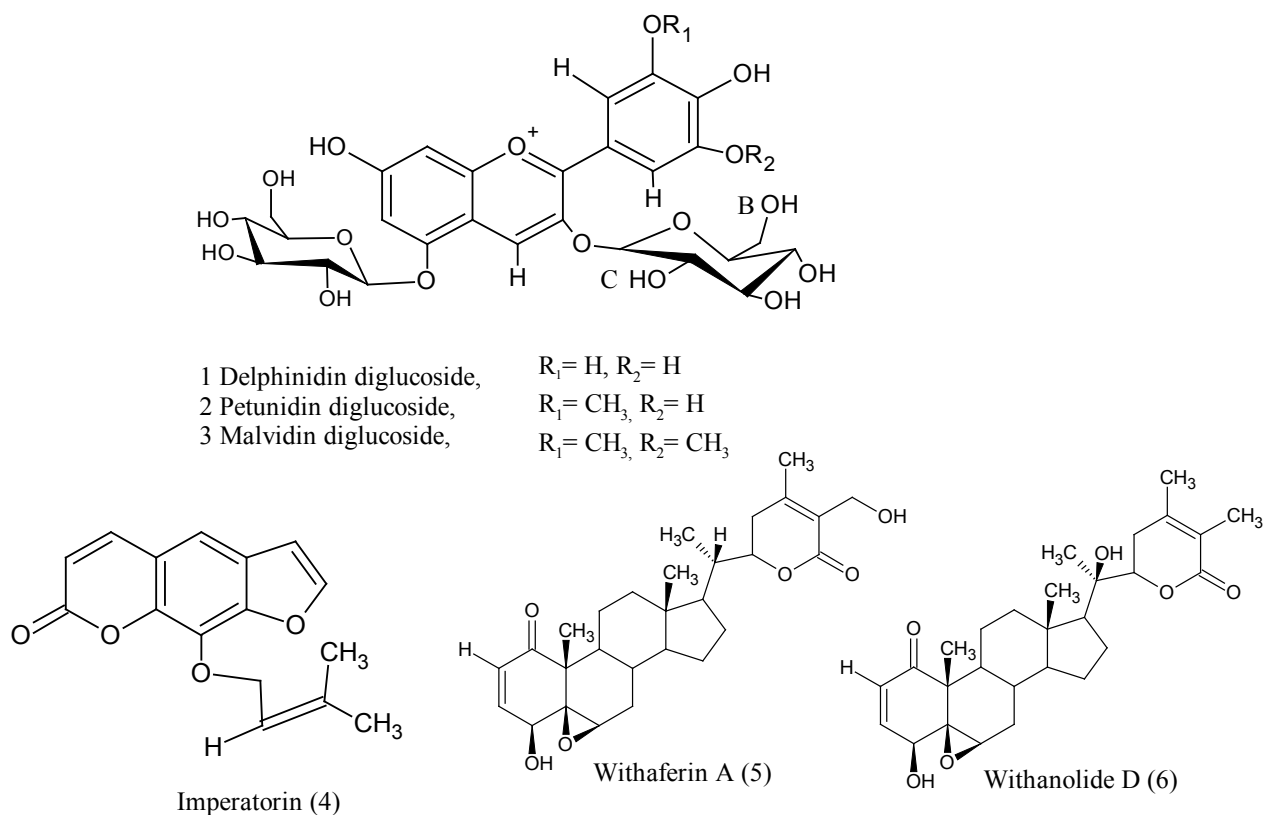


Fig. 5: Structures of marker constituents^[73]

spectra of *A. marmelos* crude extract (IS, TMB) had also shown a distinct signal for olefinic proton at δ 5.61 of imperatorin and for the three aromatic protons at δ 6.09 of TMB.

The qH-NMR gave a linear response for the marker constituents, anthocyanins, imperatorin and withaferin-A utilizing the described approach, the amount of anthocyanins in Sephadex enriched extracts and XAD of *E. jambolana* was 3.77 % and 9.57 % (delphinidin-3,5-diglucoside), 6.55 % and 15.70 % (malvidin 3,5-diglucoside), 4.72 % and 12.0 % (petunidin 3,5-diglucoside), respectively. The imperatorin content was 0.424 % in *A. marmelos* fruit and 0.090 % and 0.114 % in sharbat and candies. Total withanolides content was 0.191 % in the chloroform extract and 0.234 % in the capsule extract^[73].

Quantitative validation of paralytic shellfish toxins (PSTs) and characterization of Gonyautoxin-5 (GTX5) and Gonyautoxin-6 (GTX6):

Validation of PSTs were accomplished using INOVA 600 MHz spectrometer which was equipped with 5 mm indirect probe that has ¹H coil inside X nuclei coils with the following parameters: acquisition time of 2.2 s, 90° pulse width (9.4 min), 64 transients and relaxation delay time of 11 s. Paralytic shellfish poisoning (PSP) is a human ailment caused by consumption of crabs and shellfish contaminated with PSTs as mentioned in fig. 6.

Tertiary butanol was used as an IS which was prepared by mixing of tert-butanol/deuterated acetic acid

(CD₃COOD)/Deuterium Oxide (D₂O) with dilution (as 100 μ l/1 ml/10 g), for the quantitation validation.

The signal from the mixture tert-butanol, CHD₂COOD and deuterioacetic acid (CH₃COOD) were observed at 1.24, 2.03 and 2.05 ppm, respectively and the signal of a tert-butanol at 1.24 ppm (singlet, 9H) was used as an IS. The signals around 3.35 and 2.72 ppm might be methanol and impurity, respectively. The LOQ at 5 % RSD was calculated to be 0.16 mM, which corresponded to 67 mg/ml as Saxitoxin (STX) diacetate form, while the LOD was 0.04 mM.

GTX5 and GTX6 isolated from mussels were quantified by method and the toxicities of GTX6 and GTX5 were obtained by the Mouse Bio Assay (MBA) in which mice was standardized by STX. The particular toxicities of GTX6 and GTX5 calculated by the MBA were 105 milli-unit (MU)/mmol (25 mg STX equivalents/mmol) and 120 MU/mmol (29 mg STX equivalents/mmol), respectively^[74].

Quantitative validation of the fluoxetine:

Validation of racemic fluoxetine (S-fluoxetine and R-fluoxetine) were accomplished on Bruker DRX 500 MHz Avance spectrometer which was equipped with a dedicated 5 mm Query Node Patterns (QNP) probe with the following parameters: 90° pulses with 16-64 scans collected and the acquisition time was 0.53 s followed with a relaxation delay of 3.0 s. Quantitative ¹⁹F NMR spectroscopy was applied for the evaluation of fluoxetine enantiomers using various chiral recognition agents in pharmaceutical.

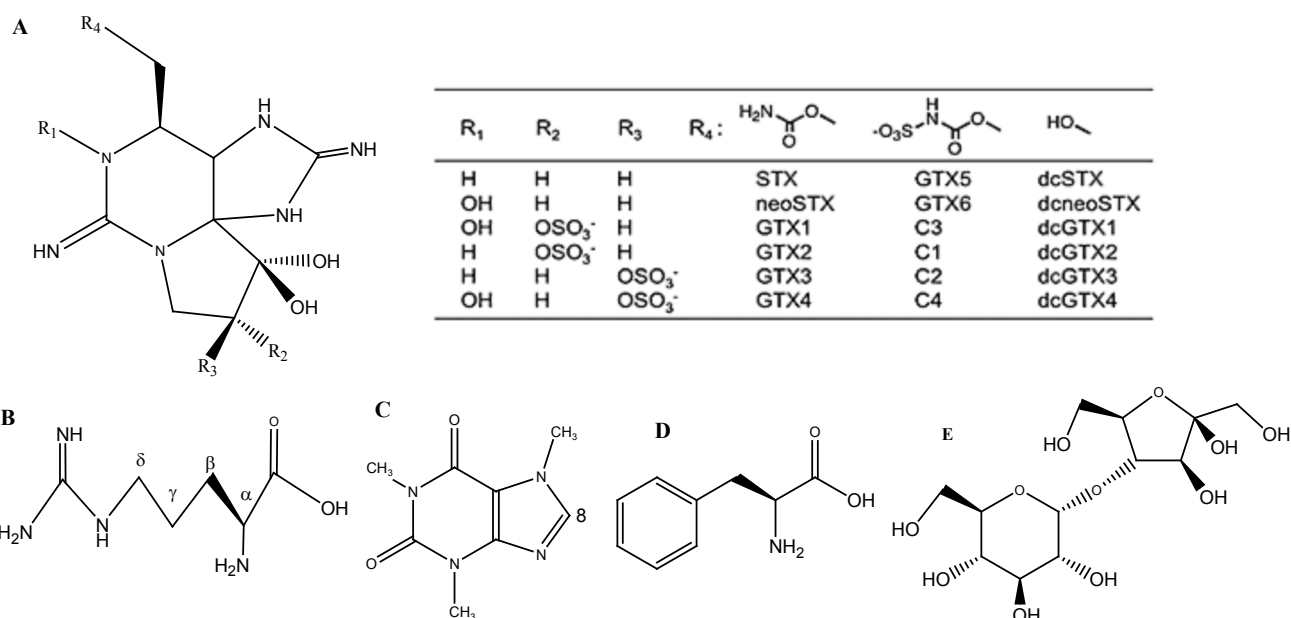


Fig. 6: Structures of a group of (A) Paralytic shellfish toxins; (B) Compounds L-arginine; (C) Caffeine; (D) L-phenylalanine and (E) Sucrose^[74]

The standard stock solutions of sodium fluoride (NaF) used as an IS and racemic fluoxetine (as shown in fig. 7) were prepared in double distilled de-ionized water by weighing 12.5 mg NaF and 25.0 mg fluoxetine. A range of 0.25-3.90 mg of diamino Derivative of Methylated α -Cyclodextrin (DAM- α -CD) was utilized as the suitable chiral selector by dissolving in diluted standard solutions into the NMR tube.

Four Cyclo Dextrins (CDs), specifically α , beta (β), gamma (γ) CD and DAM- α -CD, were utilized for enantio-separation of fluoxetine. Evaluations were performed with solutions containing 0.5 mg/ml fluoxetine and having various concentrations of CDs. The changes detected in the successive ^{19}F NMR spectra of fluoxetine upon addition of the chiral recognitions. The calibration curve was found linear for (R) and (S)-fluoxetine over the range 0.10-1.35 mg/ml, the LOD (S/N=3) being 5.9 and 7.5 $\mu\text{g}/\text{ml}$ for the pure (R) and (S)-fluoxetine, respectively.

The correlation coefficients were found >0.9980 for both enantiomers, which suggested a linear response within the studied range of concentration. The LOD was taken as the concentration of analyte where signal-to-noise (S/N)=3 and it was found to be 5.9 and

7.5 g/ml for the pure (R) and (S)-fluoxetine, respectively. The recoveries of (R) and (S)-fluoxetine from pharmaceuticals lie in between 91.2 and 109.6 % in all cases and the obtained RSD values were less than 8 %. The results showed that the method is rapid, accurate and precise^[75].

Quantitative validation of fenfluramine, sertraline and paroxetine:

Enantiomeric purity of fenfluramine, sertraline and paroxetine were validated using Bruker 500 MHz Avance spectrometer which was equipped with a 5 mm Broadband Observe (BBO) probe with the following parameters: spectra acquired with 64 transients, 30° pulse with a delay of 1 s and line broadening of 0.30 Hz was used. The evaluation of the enantiomeric purity of three Active Pharmaceutical Ingredients (APIs) were developed using the Chiral Solvating Agent (CSA) (R)-1,1'-Bi-2-naphthol (as shown in fig. 8). Chemical shifts were referenced using internal reference TMS in CDCl_3 (99 %) solvent.

Samples were prepared by weighing roughly around 1-4 mg of sample and an appropriate molar ratio of (R)-1,1'-Bi-2-naphthol (1) was added to induce separation of enantiomers when dissolved in CDCl_3 solvent.

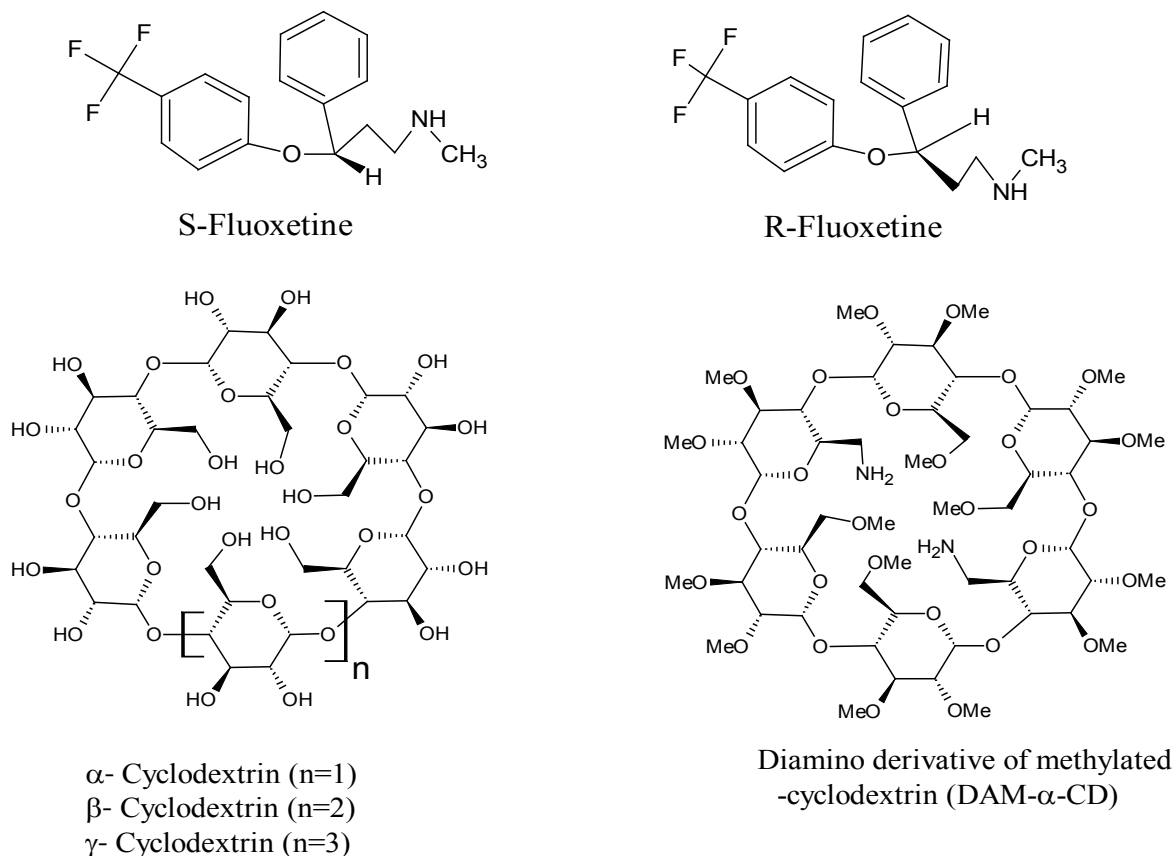


Fig. 7: Chemical structures of the racemic fluoxetine and the cyclic oligosaccharides used in the study^[75]

The resonance at ~ 2.15 ppm is due to an impurity in (R)-1,1'-Bi-2-naphthol (1) and at 1.6 ppm is due to water (2) in the spectra. The LOQ is <1 % minor component based on a percent error of <5 % at this level. The enantiomer purity of APIs were found as fenfluramine $50.2\% \pm 0.4\%$, sertraline $8.0\% \pm 0.2\%$ and paroxetine $4.5 \pm 0.1\%$. Chiral NMR using CSA was revealed to be an alternative or complimentary approach to chiral High-Performance Liquid Chromatography (HPLC) and optical rotation for the evaluation of enantiomeric purity^[76].

Quantitative validation of levofloxacin and rifampicin:

Validation of pure levofloxacin and rifampicin (as shown in fig. 9) and urine's extracts were done on JEOL-NM-LA 300 MHz, Fourier Transform NMR (FT-NMR) spectrometer with the following parameters:

delay time of 1.55 s, 500 free-induction decays (FIDs) were collected for each samples into 32 768 data points using a spectral width of 6009.6 Hz and maintained temperature of 40° throughout.

Spectra of pure drugs and urine's extracts were acquired and successive quantities of pure rifampicin (0.9 -43.0 mg) or levofloxacin (0.8-54.0 mg) were rigorously mixed with suitable quantities of maleic acid (2.0 -24.0 mg) as IS and dissolved in DMSO- d_6 .

The spectrum shows doublet at 7.57 and 7.61 ppm could be attributed to the splitting of 5H proton due to the coupling with ^{19}F nucleus at the adjacent position. The spectrum shows the NH and OH protons gave broad signals in between 12.0 and 13.0 ppm. The methoxy group (OCH_3) protons at C-37 gave a sharp singlet at 3.04 ppm whereas the methyl group (CH_3) at C-13 gave a signal at 1.85. Methylene protons at carbon 4, 5, 7 and 8 gave signals at 2.5-3.3 ppm.

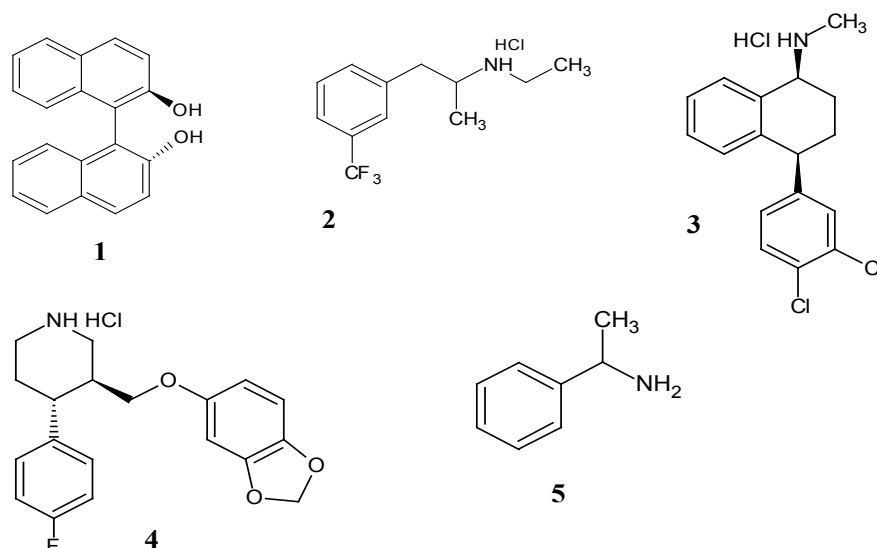


Fig. 8: Structures left to right, top to bottom: (1) (R)-1,1'-Bi-2-naphthol; (2) Racemic fenfluramine hydrochloride (HCl); (3) (+)-sertraline HCl; (4) (-)-paroxetine HCl and (5) Racemic methylbenzylamine^[76]

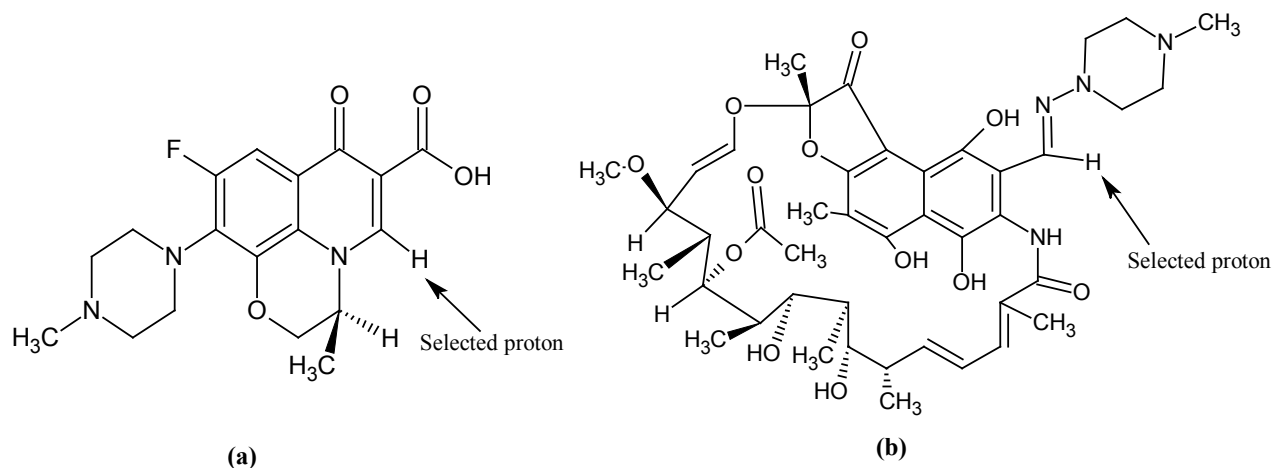


Fig. 9: Molecular structure of (a) levofloxacin and (b) rifampicin^[77]

TABLE 3: OBSERVED PARAMETERS FROM THE MENTIONED QUANTITATIVE METHOD VALIDATION

Quantitation compound	Residual solvent used (Deuterated)	IS reference used	Quantity utilized for stock solution/reference	Percentage/ RSD found
RI76	DMSO-d ₆	Acetanilide (99 %)	Acetanilide (5 mg) and (RI7612 mg)	Observed RSD values was 1.91 %
α-Bisabolol	CDCl ₃ -d	OMCTS	α-Bisabolol (105.0 mg/ml) and OMCTS (1.9 mg/ml)	Observed RSD values was <1.75 %
α-Pinene	CDCl ₃ -d	OMCTS	α-Pinene (98.0 mg/ml) and OMCTS (2.2 mg/ml)	RSD <0.76 % followed chromatographic methods (SD<2 %)
Cellulose in tobacco	As a solid used	Silicon tube	Tobacco sample Ranged from 100 to 300 mg	Observed RSD values was <1.49 %
Amygdalin	Pyridine-d ₅	HMD	HMD (10.0 mg) and bisphenol-A (10.0 mg in 1 ml stock solution)	Purity was Persicae semen 2.72 and 3.13, Armeniacae semen 3.62 and 5.19 and Mume fructus 0.23 %
Ginkgolide-B and Ginkgolide-A	DMSO-d ₅	Caffeine and DMSO ₂	DMSO ₂ (24.5 mg/ml) and Caffeine (27.5 mg/ml)	Purity was Ginkgolide-B 51.3 % and 0.45 % for Ginkgolide-A
<i>E. jambolan</i> , <i>A. marmelos</i> , and <i>W. somnifera</i>	CDCl ₃ -d	1,3,5-TMB and 2,4-diformyl phloroglucinol	TMB (1.94, 3.23 mg) and 2,4-diformyl phloroglucinol (1.42 mg)	<i>E. jambolana</i> contents 9.57 % (delphinidin), 15.70 % (malvidin), 12.0 % (petunidin) and imperatorin was 0.424 % in <i>A. marmelos</i> fruit and with anolides was 0.234 % (capsule extract)
PSTs	Acetic acid-d ₄ and water (CD ₃ COOD and D ₂ O)	Tertiary butanol	Tert-butanol/CD ₃ COOD/D ₂ O as 100 µl/1 ml/10 g	Observed RSD value was <5 % in all the cases
Fluoxetine	D ₂ O	NaF	NaF (12.5 mg) and fluoxetine (25.0 mg)	Observed RSD values was <8 %
Fenfluramine, sertraline and paroxetine	CDCl ₃ -d	TMS	TMS added in µl in CDCl ₃	Purity was fenfluramine 50.2 %±0.4 %, sertraline 8.0 %±0.2 % and paroxetine 4.5±0.1 %
Rifampicin and levofloxacin	DMSO-d ₆	Maleic acid	Maleic acid (2.0-24.0 mg), rifampicin (0.9-43.0 mg) and levofloxacin (0.8-54.0 mg)	Observed RSD values was <2.7 % for pure drugs and urine samples

Maleic acid signal at 6.2 ppm was utilized as the reference signal. Integration of signals at 8.2 and 8.9 ppm were utilized for evaluating the concentration of rifampicin and levofloxacin drugs per unit dose, respectively. Recoveries of pure rifampicin and levofloxacin were obtained (98.3-99.7)±1.08 and (97.0-99.4)±0.5 %, respectively and recoveries in urine samples were obtained 96.8-100.0. RSD values <2.7 % were observed for pure drugs and urine samples^[77].

Thus, above given examples reflects diverse methodologies used for quantification and validations which provided to be a fast and efficient alternative, providing results with less standard deviation. All evaluated parameters regarding linearity, robustness, specificity, selectivity and accuracy, as well as influences of instrument specific parameters and stability of analyte and IS in solution shown satisfactory results. The various parameters observed in above mentioned examples are given in Table 3.

CONCLUSION

NMR spectroscopy has largely given way to the most important routine analyzes of natural synthetic drug candidates and related molecules. Quality control and simultaneous quantification of different residual solvents, analytes and impurities have led analysts to further exploit NMR as a viable quantitative technique. In this review we describe the different qNMR method that is utilized for qualitative, quantitative and purity studies. The validated qNMR methods were simple, robust, precise and selective for determining different natural as well as synthetic drugs sample using several commercially accessible compounds (like TMS, maleic acid, TMB, OMCTS etc.) as IS. Since NMR quantitative analysis is suggested as a USP standard technique, the described methods can be proposed as a standard as International Council for Harmonisation (ICH) tripartite guideline. The different studied examples suggested that obtained values were in concurrence

with chromatography results directing that qNMR is as reliable as chromatography-based technique for quantitative analysis.

Conflicts of interest:

The authors declared no conflict of interest.

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