Original Article

Quantification of Langlois Reagent by ^{19}F-NMR Spectroscopy

Ganpat R. Nagargoje[a] | Abhay S. Bondge[b] | Prasad D. Kadam[c] | Kalimoddin I. Momin[d] | Sainath B. Zangade[e] | Dadasaheb D. Kadam[f] | Sharad P. Panchgalle[g] | Vijaykumar S. More[h,*]

[a]*Department of Chemistry, Shivaji Mahavidyalaya, Renapur, Dist-Latur-413527, (M.S.), India*
[b]*Department of Chemistry, Shivneri Mahavidyalaya, Shirur Anantpal Dist- Latur-41354, (M.S.), India*
[c]*Department of Chemistry, Shri Kumarswami Mahavidyalaya, Ausa, Dist -Latur-413520; (M.S.), India*
[d]*Department of Chemistry, Rajarshi Shahu Mahavidyalaya, Latur, Dist-Latur-413512, (M.S.), India*
[e]*Department of Chemistry, Madhavrao Patil ACS College, Palam, Dist- Prabhani-431720, (M.S.), India*
[f]*Department of Chemistry, Narayanrao Waghmare Mahavidyalaya, Akhada Balapur-431701, India*
[g]*Department of Chemistry, K. M. C. College, Khopoli, Dist-Raigad- 410203, India*
[h]*Department of Chemistry, Kai. Rasika Mahavidyalaya Deoni, Dist-Latur-413519, India*

Use your device to scan and read the article online

Citation G.R. Nagargoje, A.S. Bondge, P.D. Kadam, K.I. Momin, S.B. Zangade, D.D. Kadam, S.P. Panchgalle, V.S. More. **Quantification of Langlois Reagent by ^{19}F-NMR Spectroscopy.** *J. Appl. Organomet. Chem.*, **2023**, *3*(3), 213-223.

https://doi.org/10.22034/JAOC.2023.404217.1086

CC BY

Article info:
Received: 26 June 2023
Accepted: 8 August 2023
Available Online: 12 August 2023
ID: JAOC-2306-1086
Checked for Plagiarism: Yes
Language Editor checked: Yes

Keywords:
Langlois reagent, NaTFMS, ^{19}F-QNMR, Trifluoroethanol (TFE), Delay time (d1)

ABSTRACT

Sodium trifluoromethanesulfinate (NaTFMS) is also known as "Laglois reagent" developed Quantitative ^{19}F-NMR (QNMR) and also employed to determine the amounts of "Laglois reagent" in a mixture containing (NaTFMS) and other inorganic impurities, the amounts of NaTFMS can be measured by high-performance liquid chromatography, but it requires a high-purity standard sample. NMR signal was affecting the response to determined by measuring longitudinal relaxation time (T1) to be 1.2 s is the main parameter "delay time (d1) between two scans". Internal reference for the quantitative analysis was used as a trifluoroethanol of sodium trifluoromethane sulphinate by water as solvent. Six experiments with different weights of sodium NaTFMS/TFE and the relative standard deviation (R.S.D.) were repeated and the results are less than 2.0% and also detection limit of ^{19}F-QNMR upto 0.85 mg. ^{19}F-QNMR was calculated and the R.S.D.s of results were less than 2% of each mixture of amount NaTFMS.

Introduction

The scope and application of Nuclear Magnetic Resonance spectroscopy technique is continuously expanding. In conventional methods for quantitative analysis, such as chromatography, a reference Standard (RS) of a target analyte is essential to obtain accurate quantitative results which are often expensive and unavailable whereas, in the quantitative NMR technique, an External standard (ES) that differs from the target analyte is used [1] to save the cost and time of analysis. ^{1}H-QNMR, ^{13}C-QNMR, and ^{31}P-QNMR methods useful for the quantitative analysis of silicone rubber [2], blood plasma metabolites [3], algal toxins and other natural products [4], lactide composition [5], analysis of polymer sequence distribution

***Corresponding Authors: Vijaykumar S. More** (vijaymore@gmail.com)

[6], vanillin [7], coals [8], determination of deuterium isotope ratios [9], short-chain inorganic phosphate [10], purity determinations of military nerve agents [10], and agricultural chemicals [11]. Experimental precision, accuracy, specificity, linearity, and limits of detection are also proven [12]. ^{19}F-QNMR shows wider chemical shift range and higher sensitivity, and also used in the structural identification of many fluorine-containing substances [13-16].

Sodium trifluoromethanesulfinate which is also known as "Langlois reagent" is a versatile chemical used in modern days for different trifluoromethylation reactions (**Figure 1**) [17-20]. This is also one of the important precursors for the production of "Fipronil" one of the broad-spectrum insecticides [21-23]. Different chemical routes are reported for the synthesis of "Langlois reagent" [24]. However, the analytical method for purity determination of it was given by titrimetric method by using hypochlorite ($HClO_4$) as the oxidizing agent by many commercial suppliers. HPLC method requires a reference standard.

There is no ^{1}H present in the sodium trifluoromethanesulfinate. The only tool to characterize its structure is using ^{19}F and ^{13}C-NMR spectroscopy. Until now no authentic method was reported by ^{19}F-QNMR for the determination of purity and assay of "Langlois reagent". This promoted us to develop a method for ^{19}F-QNMR for determination of purity and assay of the "Langlois reagent" using water as solvent. Trifluoroethanol and "Langlois reagent" both are completely miscible in water. Therefore, we used water solution as it is for NMR analysis without dissolving D_2O, instead of that we inserted D_2O filled capillary in NMR tube for locking spectrometer. We have reduced the use of expensive deuterated solvent and saved the cost of analysis significantly. Thus, it can be used for commercial purposes. The method described here is fast and cost-effective.

All the parameters of this method like specificity, selectivity, precision, intermediate precision, linearity, the limit of detection, limit

Figure 1. Structure of (a) Langlois reagent and (b) Trifluoroethanol

of quantification, accuracy solution stability, and robustness were validated.

Experimental

Materials and methods

All reagents are pure analytical grade and used without further purifications. Sodium trifluoromethanesulfinate (98.20%) was purchased from Aldrich (742322-5G-LOT#BCBS9566V). Trifluoroethanol (99%) was purchased from Loba-Chemie (0349F00100 LOT#LL10911605).

NMR: AVIIIHD 300 MHz FT-NMR frequency at 282.44 MHz (7.1Tesla) for fluorines, fitted with a 5 mm multinuclear observes (BBO) probhead.

Procedure for the preparation of standard and test solutions

Sodium trifluoromethanesulfinate (31.2 mg) was weighed accurately for standard preparation and thoroughly mixed with trifluoroethanol (30.04 mg); it was dissolved in 0.6 ml of water and mixed completely until the solution complete dissolution.

Preparation of sodium trifluoromethanesulfinate

Sodium trifluoromethanesulfinate was weighed (15.60 mg), and then 0.6 ml of water was added to a stoppered tube till complete dissolution.

Preparation of trifluoroethanol IS preparation for specificity

IS preparation for specificity was done, trifluoroethanol (10.0 mg) was weighed accurately, transferred to the stoppered tube, and also 0.6 ml of water was added. The whole mixture was completely dissolution.

Standard preparation for robustness study (IS variation: 5.0 ±1.0 mg)

15.6 mg of sodium trifluoromethanesulfinate was taken and transferred into two different stoppered tubes and 10.05 mg and 20.10 mg of Trifluoroethanol IS was respectively added to both stoppered tubes, and then 0.6 ml of water was added and the solution thoroughly mixed till dissolution was completed.

Sample preparation

Crude trifluoromethanesulfinate (31.2 mg), trifluoroethanol (30.00 mg), and 0.6 ml of water were weighed accurately and transferred to a stoppered tube. For analysis, the solution was thoroughly mixed till dissolution was completed.

Sample preparation for robustness study (IS variation: 30.1 ± 1.0 mg)

The crude sample of trifluoromethanesulfinate were weighed, 30.0 mg and 45.0 mg of trifluoroethanol IS were transferred into two different stoppered tube, respectively, and then the solution was thoroughly mixed with 0.6 ml of water till dissolution was completed.

Confirmation of purity of trifluoroethanol (IS)

The pure trifluoroethanol was used as an internal standard for ^{19}F-QNMR spectroscopy was determined using a validated Gas Chromatographic method and by Agilent make instrument, the separation of trifluoroethanol was performed.

Procedure for ^{19}F-QNMR method

The standard preparation in replicate (n=6) and sample preparation in triplicate was performed, under the experimental conditions given as per the NMR analysis section ^{19}F-QNMR was recorded. ^{19}F signal obtained at -86.40 ppm concerning ^{19}F signal of trifluoroethanol (IS) at -76.85 ppm in water using D_2O filled capillary for locking spectrometer frequency.

Calculations

$$\% P_{sample} = \frac{I_{sample}}{I_{std}} \times \frac{N_{std}}{N_{sample}} \times \frac{MW_{sample}}{MW_{std}} \times \frac{W_{std}}{W_{sample}} \times P_{std}$$

$$W_{sample} = \frac{I_{sample}}{I_{std}} \times \frac{N_{std}}{N_{sample}} \times \frac{MW_{sample}}{MW_{std}} \times W_{std}$$

Where,

I_{sample} = Mean Integral value of the analyte (19F signal obtained at -87.4 ppm).

I_{std} = Integral value of the ^{19}F signal of Trifluoroethanol IS obtained at -76.8 ppm.

N_{std} = Number of Fluorine's for the trifluoroethanol IS.

N_{sample} = Number of Fluorine's for the analyte ^{19}F in trifluoromethanesulfinate.

MW_{sample} = Molar mass of trifluoromethanesulfinate(156.06 gm/mole).

MW_{std} = Molar mass of trifluoroethanol (100.04 gm/mole).

W_{std} = Weight of the trifluoroethanol IS.

W_{sample} = Weight of the trifluoroethanol IS.

P_{std} = Assay of trifluoroethanol (99.00%).

Results and Discussion

NMR analysis

^{19}F and ^{13}C-NMR chemical shift assignments for sodium trifluoromethanesulfinate and trifluroethanol (IS) were performed to confirm the structure of sodium trifluoromethanesulfinate. 30.2 mg of NaTFMS in 0.6 ml water and 30.0 mg of the TFE as the internal standard for quantitation were vortexed and completely dissolved internal standard. Later, clear solution was passed into the NMR tube on including a sealed capillary filled with D_2O (for locking), subsequently accreion of NMR spectra on a BrukerAvance III HD 300 NanoBay spectrometer (Bruker BioSpin, Fallanden (Switzerland) equipped with a 5mm BBO probe utilizing ICON-NMR

under the control acquired automatically. In the range of 25 to -237.0 ppm relative to CF_3Cl (δ = 0.0ppm), all ^{19}F-QNMR spectra were recorded at 300 K of the NMR probe was maintained during the entire experiment. A delay time (d1) of 20 s was acquired significantly more than 5 times T1 to ensure that full T1 relaxation and to furnish maximum signal-to-noise ratio (S/N) pulse of 90^0 angle was used and off-resonance effect of the influence was reduced on the accurateness measurement of ^{19}F-QNMR [27]. To take full advantage of sensitivity, 65536 data points over a spectral width of typically 25 to -237 ppm were collected 32 scans. The Receiver Gain (RG) was used with a 203 Bruker pulse program. All ^{19}F-QNMR spectra were automatically phased and with the help of the Topspin 3.5 software package (Bruker Bio Spin, fallanden (Switzerland) the baseline was corrected for accurate quantitative measurements. By integration of the broad regions all over diagnostic resonances, the peak areas were obtained, using an integral limit of ±20 Hz throughout the corresponding signals. Later on, the influence of the relaxation delay time, d1, on the S/N ratio of selected signals was investigated in the range of 8-30 s to attain admissible correctness and particularity mandatory for measurable determination, where the contrast of S/N was discovered in the range of 20-30 s. As a result, for a short spectra accession run time, d1 = 20 s was used for all the analysis. The FIDs had been apodised with a 0.3 Hz flourishing line enhancing function prior to Fourier transformation. Thereafter, handbook two-criterion aspect correction was used to obtain after that baseline correction a high-quality absorption line shape; for the signal integration the manual mode was used.

Determination of relaxation time T1

It is generally considered that when T1 is five times shorter than d1, nearly 99% of nuclei in the excited state can relax to the ground state. Based on this relation, by the measurement of T1, a suitable d1 value for the 1D NMR experiment can be determined. Fluorinated compounds of the longitudinal relaxation delay was determined by the inversion recovery pulse sequence method, using the t1/t2 Relaxation Bruker program which settled the data to the exponential

$$I = I_0 + P\exp(-t/T1)$$

Where, I is the intensity of the compound of interest resonance at the inversion delay time t, I_0 denotes the intensity of the compound of interest resonance at the equilibrium state, and P is a constant.

In our case, the relaxation time T1 was resolved experimentally by reverse recovery experiment for all the ^{19}F-QNMR of the Sodium Trifluoromethanesulfinate, impurities present, and internal reference standard among which the prolonged comfort moment 1.0 s was implanted for the trifluoromethanesulfinate and 1.19 s for IS with help of 30 points VD list. Accordingly, we kept significantly more 20 s delay time between pulses which was sufficient to assure fully T1 relaxation of fluorine for consistent results in water solution.

Validation of ^{19}F-QNMR

The principle of QNMR is that for a peak, whatever from the same or different molecules, its integral intensity is commensurate to the number of nuclei, i.e. the abstracted of the compound in a solution. Using this, it is simple to acquire the mole ratio of two function groups or two ingredients placed on their integrals. To the sample solution a known amount is added with internal reference, on the basis of integral area of signals from the internal reference and sample, internal reference, and target substance can be determined between the mole ratios. Hence, according to the International Conference on Harmonization (ICH) guidelines, the method was validated [26] for parameters-system suitability, specificity and selectivity, precision and intermediate precision, accuracy, range, linearity, LOD, LOQ, and robustness.

System suitability

The most important advantage of the Quantitative NMR study provides a system suitability test from the sample itself, though we have performed system precision for each criterion by reproduce acquisitions of excellence composing which was known as

system applicability test and examined the abidance of acceptance criteria as mentioned below 2 %. Three obtaining criteria that we defined were, (i) % Relative Standard Deviation (RSD) of the integral value of analyte signal should not be greater than 2.00 [27], (ii) Signal to Noise ratio (S/N) of the analyte signal should be greater than 1000 [28,29], and (iii) variation of the δ ppm value of analyte signal should not be greater than 0.2 ppm. As a result, we have found that there is no change in chemical shift after several analyses. Hence, our outcome of the system applicability was meeting the acquiring criteria at each confirmation study. This showed signs that the system was accurate and appropriate for investigation.

Specificity and selectivity

A selective study was performed by analyzing the diluent water, sodium trifluoromethanesulfinate standard preparation, trifluoroethanol IS preparation, and sample preparation. It was achieved that there was no interference at the signals attained at -76.85 ppm and -87.40 ppm for analyte Fluorine and IS discretely resultant diluents preparations (**Figure 2**).

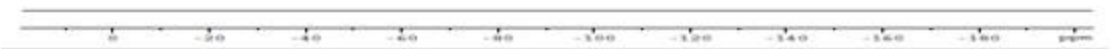
Precision and Intermediate Precision

Conforming to the ICH guidelines, the accuracy will be obtained by six repeated determinations (n=6) in addition to which moderate accuracy will be estimated by a second reviewer or/and a second NMR spectrometer through different magnetic field strength. The intermediate precision was determined by performing measurements on three different occasions. In total, six dissimilar sample preparations were prepared and analyzed on a 5 mm multinuclear BBO probe head by a disparate investigation on a disparity day. Finally, we did a moderate of six analyses, standard deviation, and relative standard deviation values which are attested in **Table 1**.

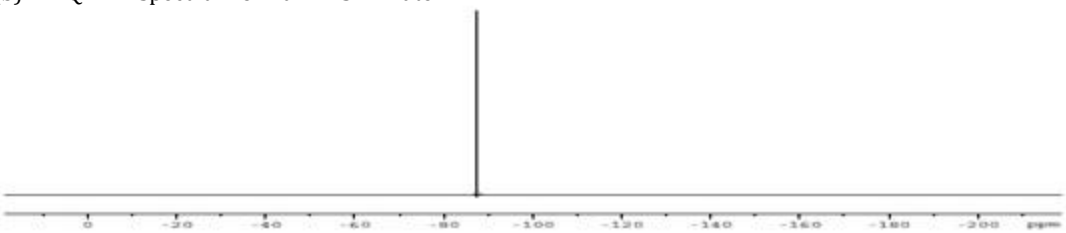
Table 1. Precision and intermediate precision test

Precision and intermediate precision test						
Precision				Intermediate precision		
Preparation	Salt taken	Salt found	%Assay	Salt taken	Salt found	%Assay
1	21.2	20.8	98.13	21.2	20.71	97.31
2	21	20.7	98.57	21.2	20.7	97.44
3	21.4	21	98.11	21.1	20.68	97.34
4	21.2	20.8	98.11	21.2	20.71	97.42
5	21	20.6	98.09	21.3	20.8	97.5
6	20.9	20.5	98.08	21.1	20.69	97.31
		Mean	98.18		Mean	97.38
		S.D	0.19		S.D	0.078
		%RSD	0.19		%RSD	0.08

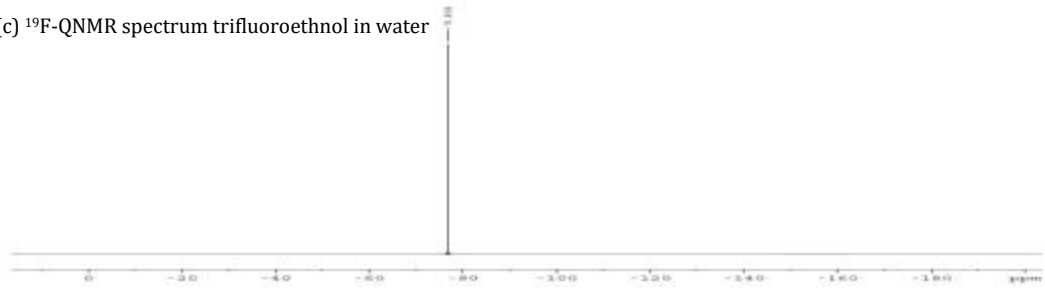
(a) ^{19}F-QNMR spectrum of diluent (water)



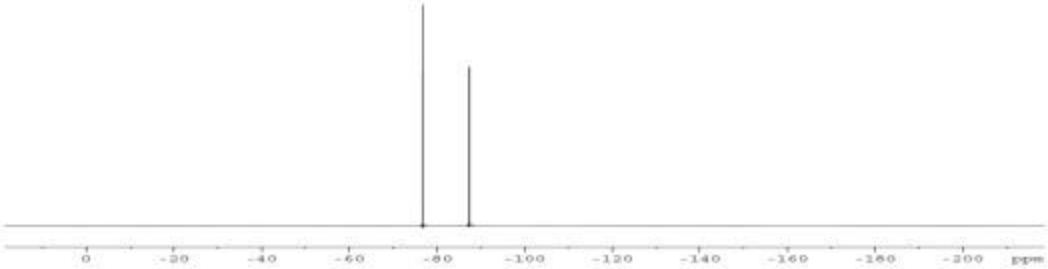
(b) ^{19}F-QNMR spectrum of NaTFMS in water



(c) ^{19}F-QNMR spectrum trifluoroethnol in water



(d) ^{19}F-QNMR spectrum standard sample preparation in water



(e) ^{19}F-QNMR spectrum sample preparation in water

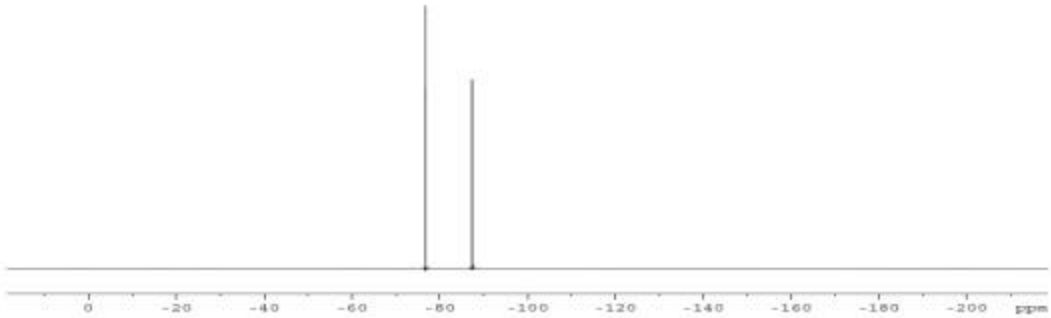


Figure 2. NMR spectrum of (a) water, (b) NaTFMS, (c) TFE, (d) standard sample mixture, and (e) sample preparation

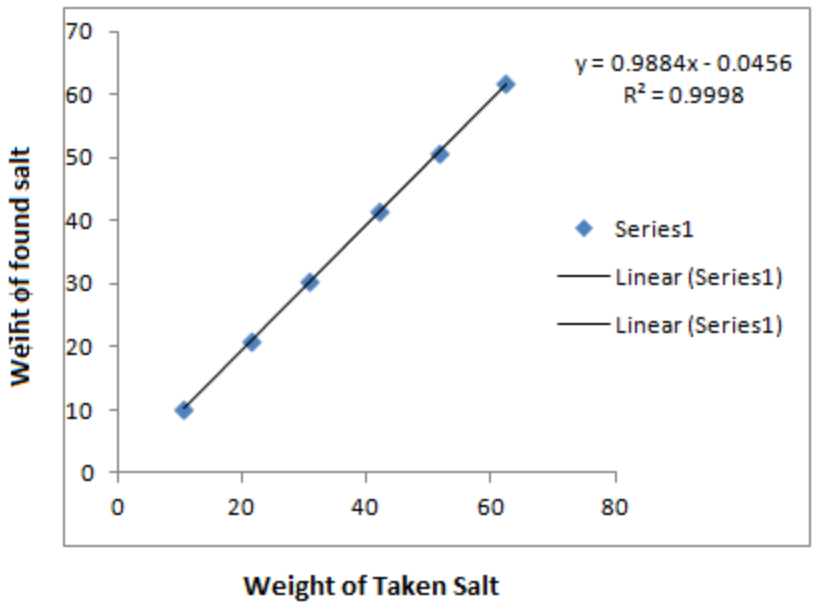


Figure 3. Linearity curve of found salt in mg vs. taken salt in mg

Linearity

The number of nuclei relating to this signal is directly proportional to the intensity of the response signal. Linearity was examined by developing standard solutions at seven dissimilar concentrations with different mole ratios by keeping in mind the content of analyte in the test sample. A curve of linearity was drawn for taken NaTFMS (in mg) vs. NaTFMS amount (in mg). The equation for the curve was y = 0.9884x - 0.0456, where the correlation coefficient was found 0.9998, that was indicating good linearity (**Figure 3**).

LOD and LOQ

In the matter 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 acquired in Linearity study. Values that had been found were 0.85 mg and 2.6 mg per ml of diluent for LOD and LOQ, respectively.

Range

Concerning the case of dimension study, it was resolved by preparing solutions of the drug up to saturated concentration in solution. At this moment, the saturated solution was prepared by adding excess drug amount and the dissolved concentration of the drug be analyzing supernatant solution. Data that had been found corresponding to saturation concentration was 287 mg per 0.60 ml diluent.

Accuracy

According to ICH documents it is recommended that accuracy should be evaluated by employing of nine determinations over three concentration levels, layering the specified range (i.e. three concentrations and three replicates of each concentration). Data as of nine determinations over three concentration levels layering was determined as the specified range. Later, the accuracy was studied at different levels i.e. 80%, 100%, and 120% relating by preparing the solutions in triplicate at each level to the sample. Hence, an analytical procedure should be established across its range for the accuracy. According to **Table 2**, it was decided that the method for assay content was accurate between the ranges of level i.e. 80% to 120%. Likewise, %RSD at each level was found to be less than 2.00.

Table 2. Accuracy test results

Accuracy Test Results *			
Accuracy Level %	**Wt. of Salt Taken**	**Wt. of Salt Found**	**Assay**
80	16.2	15.7	96.91
80	16.4	15.8	96.31
80	16.2	15.8	97.53
100	21.2	20.5	96.69
100	21.3	20.7	97.18
100	21.4	20.7	96.72
120	25.2	24.4	96.82
120	25.4	24.6	96.85
120	25.3	24.6	97.23
		Mean	96.92
		SD	0.36
		RSD	0.38

*All samples were repeated three times.

Stability of analyte in solution

Stability of analytes (and standard) on top of the analysis period showed that that the system under test should not alter during the test if the results from the test are going to be meaningful concerning the original sample. The solution is mentioned to be stable in the assay if the % difference is not more than 1.0 when kept side by side to the initial value. The standard preparation and sample preparation were examined at ambient temperature (~25 °C) during specific intervals that are 0 (initial), 6, 24, 48, and 72 hrs, and then calculated their % assay for all intervals. Calculated % difference for both the preparations at different time intervals was related to the corresponding initial value and found that it had no major change. Their results are tabulated in **Table 3**.

Table 3. Stability study

Stability of Test Solutions*								
	Standard				Sample Preparation			
Time (hrs)	NaTFMS Taken	NaTFMS found	%Assay	Difference	NaTFMS Taken	NaTFMS found	%Assay	Difference
0	21.2	20.8	98.11		21.2	20.71	97.31	
6	21.2	20.91	97.64	0.47	21.2	20.7	97.44	0.13
24	21.2	20.75	98.58	0.47	21.2	20.68	97.34	0.03
48	21.2	20.85	98.35	0.24	21.2	20.71	97.42	0.11
72	21.2	20.82	98.2	0.09	21.2	20.8	97.5	0.19

*All samples were repeated three times.

Table 4. Robustness study

Robustness Study						
Sample Preparation						
Parameters	Number of Scan			Internal Standard(mg)		
	8	16	64	10	20	40
Found salt	20.8	20.87	20.78	20.58	20.65	20.49
Assay	98.11	98.44	98.06	97.07	97.4	97.5
Difference	No difference	0.33	0.05	0.48	0.11	No difference

Robustness

The robustness of the method was analyzed by changing criterion separately: (1) The Internal standard amount (30 mg ± 1) and (2) The number of scans (64 scans ± 16) and all samples were prepared fresh daily. The estimated amount of NaTFMS appreciably did not change after clearing the100% internal standard amount. An analytical procedure of robustness and its capacity to stay unchanged by a minute, but the procedure documentation listed procedural parameters are variations deliberated and impart an evidence of its applicability during normal usage. After running the experiment using a different number of scans that are 8, 16, and 80 rather than 64 also did not have an effect on the measurement (**Table 4**).

Comparison with other technique (HPLC)

Assay results that had been acquired by comparing QNMR with other in-house HPLC techniques were also confirmed. The results of the HPLC method with QNMR did not show any noticeable differences were recognized. Also, they did not show any anomalous differences with method precision and intermediate precision. The accuracy of an analytical method indicates the adjacency of compliance between sequences of analysis developed from several sampling of the identical homogenous sample. On the Signal to Noise ratio (S/N) of the signals of interest, the precision of the integration procedure of QNMR is dependent. S/N of as minimum as 450:1 is essential for each resonance line; which should be integrated, for a accuracy better than 99% or uncertainty of 1% [27,]. We have found the exact assay of the Standard sample, which was reported in COA by the Manufacturer by our method.

Conclusion

^{19}F-QNMR was developed and hired found fast as well as simple to implement, cost-effective. The performances of the method of our satisfied requirements are different aspects, such as linearity, precision and accuracy. Sodiumtrifluoromethane sulphinate of routine quality control analysis can be used for previously described procedures of offers an excellent choice. Assay conclusions obtained by ^{19}F-QNMR were proved by equating with in-house HPLC and titrimetric method.

Orcids

Ganpat R. Nagargoje
https://orcid.org/0009-0003-3796-8496
Abhay S. Bondge
https://orcid.org/0000-0002-6249-7185
Prasad D. Kadam
https://orcid.org/0009-0004-1480-2271
Kalimoddin I. Momin
https://orcid.org/0000-0001-6297-6509
Sainath B. Zangade
https://orcid.org/0000-0002-9659-3458
Dadasaheb D. Kadam
https://orcid.org/0009-0004-1480-2271
Sharad P. Panchgalle
https://orcid.org/0000-0001-9706-7567
Vijaykumar S. More
https://orcid.org/0000-0002-6110-8774

Acknowledgements

We are gratefully acknowledged Dr. Sandeep More, Scientist (Institute of Chemical Technology, Mumbai, India) for fruitful discussions.

References

[1]. J.L. Jungnickel, J.W. Forbes, *Anal Chem.,* **1963**, *35*, 938–942. [Crossref], [Google Scholar], [Publisher]

[2]. C.C. Liu, G.E. Maciel, *Anal. Chem*., 1**996**, *68*, 1401–1407. [Crossref], [Google Scholar], [Publisher]

[3]. R.A.D. Graaf, K.L. Behar, *Anal. Chem*., **2003**, *75*, 2100–2104. [Crossref], [Google Scholar], [Publisher]

[4]. I.W. Burton, M.A. Quilliam, J.A. Walter, *Anal. Chem*., **2005**, *77*, 3123–3131. [Crossref], [Google Scholar], [Publisher]

[5]. K.A. M. Thakur, R.T. Kean, E.S. Hall, M.A. Doscotch, E.J. Munson, *Anal. Chem*., **1997**, *69*, 4303–4309. [Crossref], [Google Scholar], [Publisher]

[6]. M.R. Seger, G.E. Maciel, *Anal. Chem*., **2004**, *76*, 5734–5747. [Crossref], [Google Scholar], [Publisher]

[7]. E. Tenailleau, P. Lancelin, R.J. Robins, S. Akoka, *Anal. Chem*., **2004**, 76, 3818-3825. [Crossref], [Google Scholar], [Publisher]

[8]. A. Jutkiewicz, G.E. Maciel, *Anal. Chem*., **1995**, *67*, 2188–2194. [Crossref], [Google Scholar], [Publisher]

[9]. I. Billault, R. Robins, S. Akoka, *Anal. Chem*., **2002**, *74*, 5902–5906. [Crossref], [Google Scholar], [Publisher]

[10]. D.R. Gard, J.C. Burquin, J.K, Gard, *Anal. Chem.*, **1992**, *64*, 557–561. [Crossref], [Google Scholar], [Publisher]

[11]. T.J. Henderson, *Anal. Chem*., **2002**, *74*, 191–198. [Crossref], [Google Scholar], [Publisher]

[12]. G. Maniara, K. Rajamoorthi, S. Rajan, G.W. Stockton, *Anal. Chem*., **1998**, *70*, 4921–4928. [Crossref], [Google Scholar], [Publisher]

[13]. G.L. Abbott, G.E. Blouse, M.J. Perron, J.D. Shore, L.A. Luck, A.G. Szabo, *Biochemistry*, **2004**, *43*, 1507–1519. [Crossref], [Google Scholar], [Publisher]

[14]. A.B. Shtarov, P.J. Krusic, B.E. Smart, W.R. Dolbier, *J. Am. Chem. Soc*., **2001**, 23, 9956–9962. [Crossref], [Google Scholar], [Publisher]

[15]. S. Mele, A. Chittofrati, B.W. Ninham, M. Monduzzi, *J. Phys. Chem*., **2004**, *108*, 8201–8207. [Crossref], [Google Scholar], [Publisher]

[16]. M. Takasaki, K. Kimura, K. Kawaguchi, A. Abe, G. Katagiri, *Macromolecules,* **2005**, *38*, 6031–6037. [Crossref], [Google Scholar], [Publisher]

[17]. B.R. Langlois, E. Laurent, N. Roidot, *Tetrahedron Lett.,* **1991**, *32*, 7525–7528. [Crossref], [Google Scholar], [Publisher]

[18]. C. Zhang, *Adv. Synth. Catal*., **2014**, *356*, 2895–2906. [Crossref], [Google Scholar], [Publisher]

[19]. M.B. Swami, G.R. Nagargoje, S.R. Mathapati, A.S. Bondge, A.H. Jadhav, S.P. Panchgalle, V.S. More, *J. Appl. Organomet. Chem.,* **2023**, *3*, 184-198. [Crossref], [Publisher]

[20]. B.R. Langlois, E. Laurent, N. Roidot, *Tetrahedron Lett*., **1992**, *33*, 1291-1294. [Crossref], [Google Scholar], [Publisher]

[21]. L.R. Hatton, E.W. Parnell, D.A. Roberts, *N*-Phenylpyrazole derivatives, *US Patent,* **1985**, No. 4496390. [Crossref], Publisher]

[22]. L.R. Hatton, E.W. Parnell, D.A. Roberts, **1985**, 4-Trifluoromethylphenylhydrazinomethylene Malononitriles, *US Patent* **1985,** No. 4541963. [Crossref], [Publisher]

[23]. G. Jadhav, P. Kadam, G. Nagargoje, A. Bondge, S. Jadhav, V. More, *World J. Pharm. Res.,* **2023**, *12*, 264-281. [Crossref], [Google Scholar], [Publisher]

[24]. Y. Yingda, Encyclopedia, Eros *encyclopedia of reagents*, **2014**. [Crossref], [Publisher]

[25]. F. Malz, H. Jancke, *J. Pharm Biomed. Anal*., **2005**, *38*, 813-823. [Crossref], [Google Scholar], [Publisher]

[26]. ICH Harmonized Tripartite Guidelines, Validation of Analytical Procedures: Text and Methodology Q2 (R1), **2005**. [Crossref], [Google Scholar]

[27]. H. Gadape, K.S. Parikh, *J. Chem. Pharm. Res*., **2011**, *3*, 649-664. [Crossref], [Google Scholar], [Publisher]

[28]. W. He, F. Du, Y. Wu, Y. Wang, X. Liu, H. Liu, X. Zhao, *J. Fluor. Chem.*, **2006**, *127*, 809–815. [Crossref], [Google Scholar], [Publisher]

[29]. D.S. Kadam, S.G. Patil, D. Mammen, S.D. Kadam, V.S. More, *J. Appl. Organomet. Chem.,* **2023**, *3*, 13–27. [Crossref], [Google Scholar], [Publisher]

Copyright © 2023 by SPC (Sami Publishing Company) + is an open access article distributed under the Creative Commons Attribution License (CC BY) license (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.