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Quantification of Drugs by Proton NMR Incorporated Internal Standard Method

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Abstract

Quantitative Proton NMR (**qNMR**) by using internal standard method is reported for quantification of drugs. 1, 4-Bis-Trimethylsilanyl-benzene is used as an internal standard for quantification of some commonly used drugs. It resulted in good quantitative estimation, and complies less than 1% with HPLC. An effort towards substantial advantageous application of quantitative NMR is reported.

Keywords:

1, 4-Bis-Trimethylsilanyl-benzene, qNMR, Drug purity Y.

1. Introduction

NMR is one of the most important and widely used analytical tool in academic [1] and industrial research [2]. It enables a unique and, in principle, qualitative and quantitative determination of the relative amount of molecular groups, thus offering a tool to quantify entire molecular structure even in mixtures [3]. According to **US** pharmacopeia the NMR spectroscopy can be used for the qualitative as well as quantitative estimation of the drugs. The first qNMR had been described in the literature in 1963 by Jungnickel and Forbes. [4] After this particular focus on quantitative measurement is made for the estimation of the drugs [5-7]. Malz et. al. and other showed that it is validated technique for the quantification [8]. qNMR is an attractive, viable alternative tool to verify the purity of compounds other than conventional time consuming chromatographic techniques. Furthermore qNMR spectroscopy can be used as arbitrary tool, prior develop method for the quantification by LCMS –HPLC techniques. Thus there is always demand of other than chromatographic analytical methodology for qualitative and quantitative estimation. Previously numerous internal standards (SI) were studied for the quantification purpose [9] and only unique applications of each SI were reported [10-11] but very few of them meets the demand of suitable SI so as to resolve this issue. A suitable SI should have ability of providing unique and stable signal, solubility in commonly used NMR solvents, non volatile, non reactive, long term stable, easily weighable, nonhygroscopic, having optimum molecular weight as small molecular weight analyte need less quantity of SI. During the literature survey we found that 1, 4-Bis-Trimethylsilanyl-benzene ([TMS] $_2$Ph) can meet all these criteria and it may be used widely for quantification purpose of organic compounds [12]. Our further selection criteria for the SI were least reactivity towards the analyte/solvent (inertness), protolytic properties, purity, toxicity and suitability for multi drug analysis. The NMR can be complimentary or moreover alternate analytical tool over chromatographic techniques such as HPLC, GCMS, and LCMS

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for quantification purpose. The chromatographic methods were used for long time but it involves tedious process in terms of time, methods, consumables etc. NMR spectroscopy can be used to determine the origin of raw material and the production method of drugs, in particular, to calculate the purity of drugs at the preliminary stage of the complete drug analysis.

The pharmacologically active metabolites are often hampered by the unavailability of quantitative information in primary stage, and lot of time and consumables (solvents, columns etc.) are required in developing the method by LCMS-HPLC morever, it needs special method for particular drug. These facts could be the most important aspects to find out easy quantifying method of analysis. Keeping in mind all these things we initiated the study of some commonly used drugs by qNMR.

2. Experimental

2.1. Materials and Methods

2.1.1. Chemicals

NMR solvent Dimethylsulfoxide-d6 (DMSO-d6) (Sigma-Aldrich, 99.9% D); $CDCl_3$ (Eurisotop, (99.8%D), Methanol-d4 (Sigma-Aldrich, 99.9%D); 1, 4-Bis-Trimethylsilanyl-benzene (SI) (99.9% pure, certified reference material) was purchased from J&K Scientific Ltd. and Acetonitrile (Merck, 99.9% pure), Dimethylsulfoxide (Merck,99.99% pure), Methanol (Ranbaxy, 99.98% pure) (All HPLC Grade) was used as received. The drugs (as shown in Fig.1) 1a, 1b, 1d were procured from Alice healthcare Pvt. Ltd. India, with certified purity more than 99.0%. 1c, 1e, 1f, (Sigma-Aldrich, > 99.5%), and 1g was purchased from Merck, India with 100% purity.

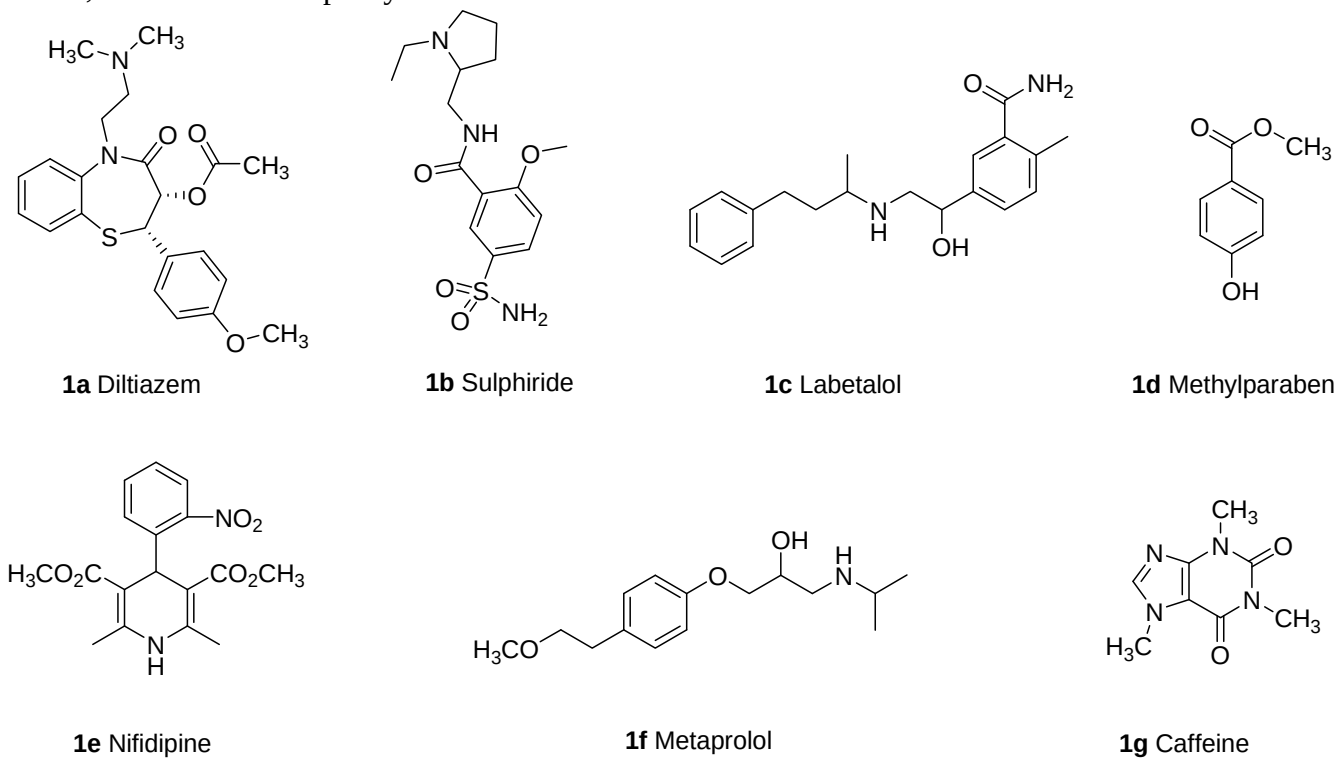

Fig1. Structures of the drugs used for the assessment of the qNMR.

2.1.2 Instrument

2.1.2.1 NMR

The proton NMR spectrum was measured with a Varian FT-NMR 400MR spectrometer at operating frequency 399.8233 MHz, ambient temperature, and conventional 5mm NMR tube. Probe: 400ASW PFG 4NUC/40-162 MHz, other conditions used were data points 32 K, lock material was used as solvent, spectral width 10000.2 Hz for all samples, pulse width 12.200 µsec (pulse angle 90°), pulse delay time 1 sec, and number of FID accumulation 36.Total time for acquisition was approximately 3 minutes, line broadening 0.3 Hz. FID processing was done by VNMRJ-2.2C-Chempack-4 software. FT-NMR spectra were recorded of pure solvents prior to analysis of standard and sample solution.

2.1.2.2 Chromatographic conditions

An Agilent 1100 series HPLC system consisting of a quaternary pump, a G1322A degasser, a G1313A auto sampler, a G1316A thermostatted column compartment, and a PDA detector (Agilent, Palo Alto, USA) was used to achieve HPLC chromatograms. The chromatographic separation for 1a, 1b, 1c and 1f was carried out on a C_{18} Waters Novapak column (50mm×2.1mm×1.8µ) maintained at 30°C fitted with a Waters Nova-pak guard column (3.9 mm×20 mm). The mobile phase: ACN: Water (90:10) (A) + 10 mM Ammonium acetate (pH=7.4) buffer (B). Run time 6.5 min with gradient program 0.0–0.01 min, linear gradient 4% B; 0.01-0.5 min, linear gradient 4% B; 0.5-3.5 min, linear gradient 95% B; 3.5-4.5 min, linear gradient 95% B; 4.5-5.5 min, linear gradient 95% B; 5.5-6.5 min, linear gradient 4% B; at a flow rate of 0.8 mL min^{-1}. The UV absorbance was monitored at 230 nm using PDA detector. Injection volumes 5 µL. For 1d,1e (same HPLC and solvent system and column dimensions) the run time was 3.5 min, gradient program was 0.0-0.1 min, linear gradient 100% A; 0.1-0.2 min, linear gradient 100% A; 0.2-2.6 min, linear gradient 100% B, 2.6-2.9 min, linear gradient,100% B, 2.9-3.1 min. linear gradient 100% A,3.1-3.5min, linear gradient 100% A. For the sake of convenience each of 100 ppm chromatogram was selected for study. Row data was processed by Class-VP software. High purity water was prepared by use of Millipore (Bedford, MA, USA) Milli Q plus water purification equipment. (The detailed procedure of method development and results for HPLC is not discussed in this paper).

2.2. Procedure

For NMR all samples were freshly prepared at ambient temperature. A standard stock solution of **SI** 2.7 to 5.2 mM in three commonly used solvents such as DMSO-d6, MeOD, $CDCl_3$ were prepared individually. These solutions were used further for making samples. Beforehand, from stock solutions 600 µL blank solvents were taken and subjected to run standard ($[TMS]_2Ph$) qNMR. The 15.5-40 mM range of above samples were dissolved in 600 µL solvents, in 1 ml sample vial sonicated for 3 min. to get clear solution then transferred it into NMR tube for the analysis. The reference was given to solvent residual peaks described by Gottlieb et. al. [13]

2.3 Calculation of purity by NMR

Sample composition, excitation pulse angle, NMR tube size, sample volume, Stringent control of the parameters as well as apodization by the window function, zero filling and phase, baseline and drift-corrections helps to achieve an accurate quantification. Traditionally the Integrated peak area is used for quantification purposes but, the peak height might also be very useful, provided that the normalization of the width-at-half-height of the internal standard peak (set at, for example, 3 Hz) through incremental adjustment of Gaussian or Line-broadening apodization [14] is done. The intensity of the NMR spectrum peaks is directly

dependent on number of nuclei present in the sample, the relations given below may be use to calculate purity; the detailed calculations are described elsewhere [15].

$$P_{analyte} = I_{analyte} \bullet \frac{N_{SI}}{N_{analyte}} \bullet \frac{M_{analyte}}{M_{MS}} \bullet \frac{m_{SI}}{m_{analyte}} \bullet P_{SI}$$

There are two ways by which the purity of the analyte can be calculated by qNMR method either by considering entire integration of the spectrum (omitting solvent peaks integration) or by individual peak integration. The SI resonates at 0.22 and 7.48 ppm in form of two sharp singlets. Rare of the drug signals overlap on upfield signal of the SI while overlap with 7.48 ppm is commonly observed.

3. Results and discussion

Regardless of the equipment cost, the use of NMR spectroscopy to identify pharmaceuticals with a high sample volume can be commercially more feasible than identification by HPLC-LCMS because of the low cost of a single proton spectrum and consumables, the short acquisition time required to record a spectrum etc. For example, if a PMR analysis can be completed in 2 min and it requires 10 min. to analyze by chromatographic methods, then the costs of the analysis is same even if the cost of the NMR equipment is an order of greater magnitude. Table 1 exhibits the values of the purity which were calculated from the ratio of a particular peak relative to that of ([TMS] $_2$Ph). Inspection of Table 1 reveals that comparable results were obtained to that of purity obtained by HPLC, however in case of the compound **1d** it shows little bit more deviation from the HPLC purity.

The chromatogram shown in Fig.2 indicates 0.28% impurity observed at 3.88 min. can be also seen in qNMR (Fig.3) shown by arrow.

The experiments were performed with analyst having no prior knowledge of analyte concentration or chemical structure. The samples were analyzed with zero spin to avoid spinning side bands in the spectrum, which directly affect the integration ratio; to overcome this difficulty the gradient shimming (field homogenization) was also performed.

The drugs for analysis were selected randomly so that the method can be applied to any kind of drugs depending upon solubility in the given solvent, choice of suitable solvent also plays key role in the quantification, therefore we tried three commonly used solvents, and fortunately all samples were completely soluble at given concentration in these solvents, however some solvent effects have been observed like in case of sample **1c,** which is completely insoluble in $CDCl_3$.

Table 1. Purity comparison by NMR and HPLC.

Compoud	% NMR Purity Purity			
	DMSO-d6	MeOD	$CDCl_3$	% HPLC
1a	99.60	99.42	98.94	99.71
1b	99.75	99.65	98.43	99.17
1c	98.98	98.17	Insoluble	99.69
1d	96.79	99.27	99.85	100.00
1e	98.25	99.37	99.03	99.77
1f	98.86	98.40	99.98	99.25
1g	99.14	98.97	99.66	------

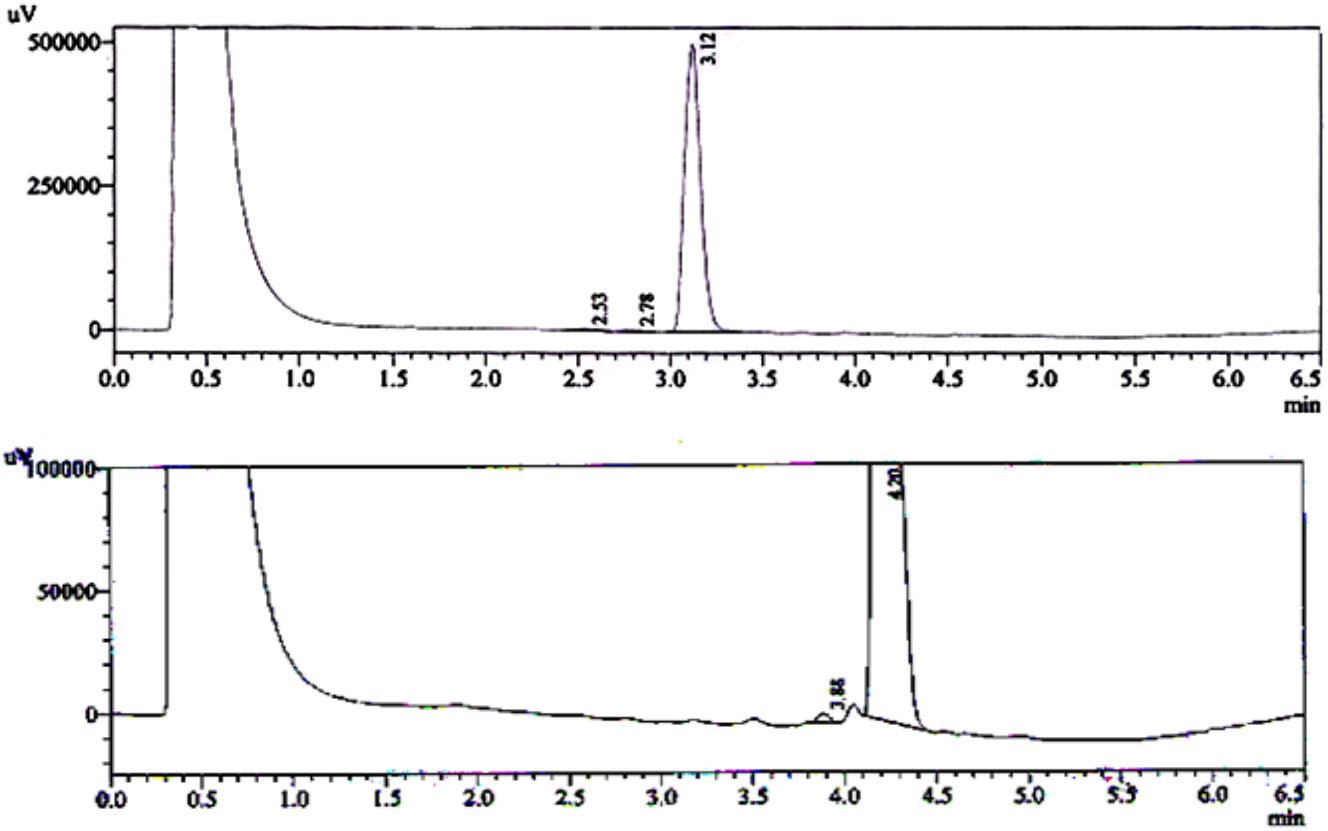

Fig.2 Typical chromatogram obtained from 1f and 1a respectively, in 1a chromatogram spiked with 0.28 % impurity at 3.88 min. showing the resolution is achieved.

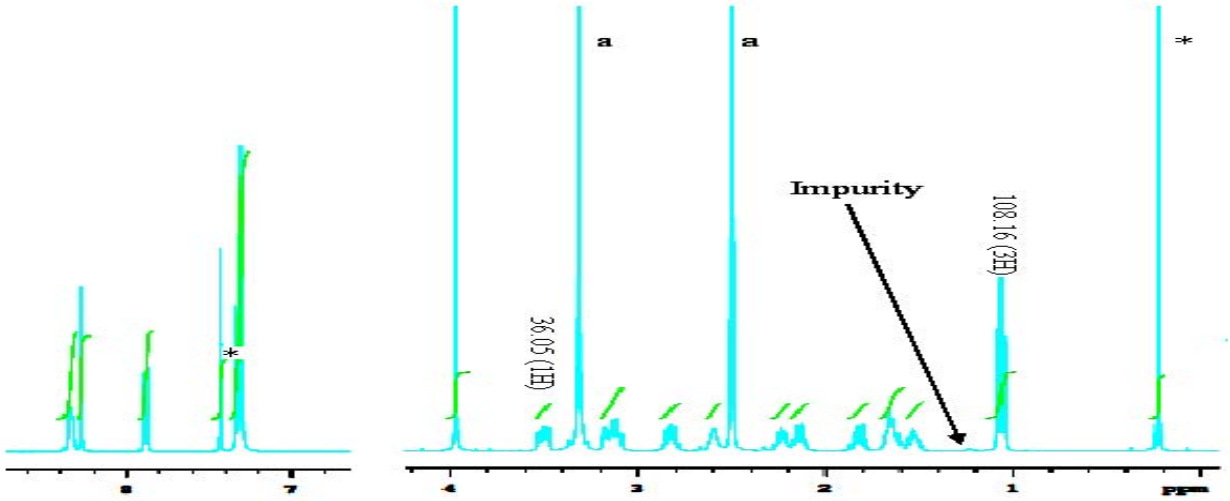

Fig.3 Two portions qNMR spectrum of 1a. Isolated peaks were used for purity calculations. The aromatic, upfiled signals of ([TMS]$_2$Ph) and solvent residual peaks are marked by 'asterisk' and 'a' respectively**.**

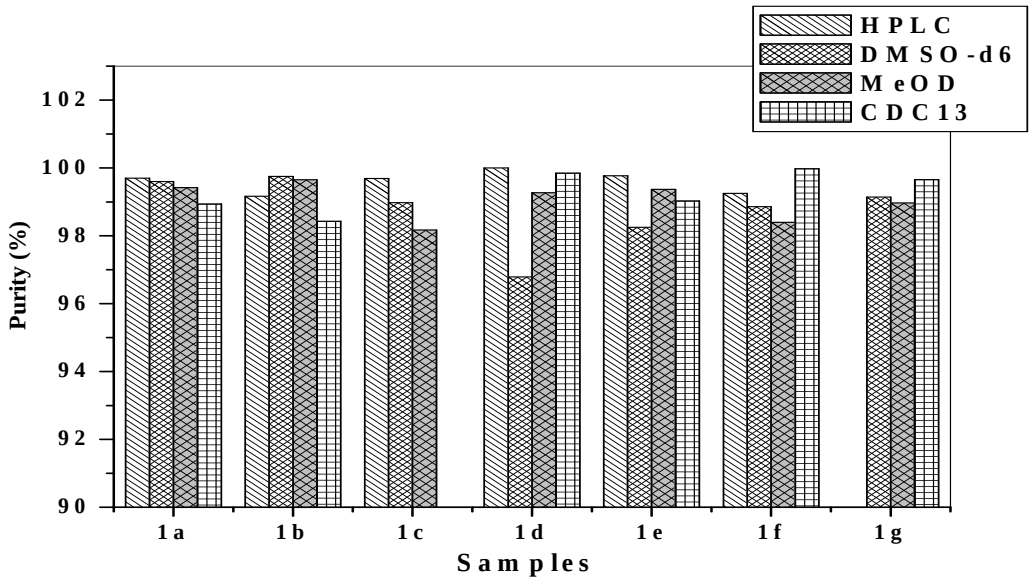

Fig.4 Graphical representation of purity comparison by solvent

From the **Fig. 4** one can understand that commonly used better solvent may be DMSO-d6 for the quantification of these drugs. It is also interesting to note that for drug **1d,** the HPLC percent purity is 100.00 while the percentage NMR purity by using DMSO-d6, MeOD and $CDCl_3$ are 96.79, 99.27 and 99.85 respectively. This may be due to the fact that the impurities which are soluble in NMR solvents may not be soluble in HPLC solvent. Thus, NMR gives better idea about the presence of impurities in drug molecule than HPLC under appropriate conditions.

4. Conclusions

The proposed internal standard (1, 4-Bis-Trimethylsilanyl-benzene) is suitable for quantification of drugs. Implementation of above qNMR method to qualify the generated samples as analytical standards can circumvent for drugs. Solvents, fine chemicals, agrochemicals, Pharma intermediates and reaction intermediates etc. can be quantified by qNMR method and may reduce the chromatographic efforts. NMR gives better idea about the presence of impurities in drug molecules than HPLC under appropriate conditions. Further, development in qNMR would be superior to the HPLC in future.

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