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Quantitative determination of mebeverine HCl by NMR chemical shift migration

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ABSTRACT

Quantitative ^{1}H NMR spectroscopic methods are not frequently reported, but current NMR instrumentation allows ready access to such data. Mebeverine HCl is an attractive molecule for NMR spectroscopy teaching purposes as it possesses a variety of simple but significant functional groups; we fully assign its ^{1}H and ^{13}C NMR spectra. Using mebeverine HCl, we show that concentration changes, in water as a solvent, can lead to significant changes in the ^{1}H chemical shifts of non-exchangeable aromatic protons and to a lesser extent to aromatic methoxy protons. An important observation is that different protons migrate to different extents as the concentration of the solute is varied, and thus the ^{1}H NMR spectra are concentration-dependent across a useful range. This chemical shift variation of selected protons was therefore analyzed and applied in the quantitative determination of mebeverine HCl in a medicine (Colofac IBS) formulated as a tablet. Self-association phenomena in water could account for these observed chemical shift migration effects as shown by determining the hydrodynamic radius from the modified form of the Stokes–Einstein equation, and thence the apparent hydrodynamic volume, V_H, for mebeverine HCl in D_2O solution which is 10-fold greater than that seen in either $CDCl_3$ or CD_3OD.

1. Introduction

Whilst ^{1}H NMR spectroscopy is well established for the identification of a wide range of compounds, the use of quantitative NMR spectroscopy is increasing as the simultaneous determination of both identity and quantity is attractive. Chemical shifts report on the chemical surroundings of atoms; changes in shifts have been extensively evaluated to monitor inter- and intramolecular interactions occurring through covalent or non-covalent bonds. In 1998, unprecedented quinoline concentration-dependent chemical shift variations in ^{1}H NMR spectra were reported,[1] and this was recently applied (as the negative decimal logarithm of concentration) to their quantitative determination.[2] Such chemical shift variations can be so pronounced that they lead to 1D and 2D spectroscopic signatures that may be misleading.[1] Lack of consideration of the importance of such concentration effects can lead to unwanted controversies in the design of self-replicating systems.[3–5] Chemical shifts have been studied to characterize aggregation,[6] hetero-[7] or self-association of chlorpromazine[8] and cromolyn sodium,[9] molecular recognition phenomena,[10,11] and to calculate association constants.[12,13]

The use of NMR spectroscopy for many analytical purposes,[14,15] including the quantitation of drugs by means of the average integral intensities of selected ^{1}H NMR spectroscopic signals compared with those of external or internal standards,[16–21] is well known. However, validation of quantitative NMR spectroscopic assays is required as a significant interlaboratory effect was found confirming the difficulty of obtaining very precise analytical data by integration of complex NMR signals.[22] Overall, from an industrial pharmaceutical analytical perspective, ^{1}H NMR spectroscopic techniques still remain quite insensitive when compared with current MS, UV, and HPLC techniques. Here we report a robust, quantitative NMR spectroscopic method.

Mebeverine HCl, (*RS*)-4-[ethyl(4-methoxy-α-methylphenyl) amino]butyl veratrate hydrochloride,[23] is an antispasmodic with direct action on the smooth muscle of the gastro-intestinal tract. It is used in conditions such as irritable bowel syndrome (IBS),[24] sold over-the-counter as Colofac IBS in the UK and as Spasmotalin and Colospasmin forte in Egypt. Mebeverine HCl also has sufficient complexity within its simple structure to be an ideal teaching tool for ^{1}H and ^{13}C NMR spectroscopy with its benzoate ester, tertiary amine, chiral center (albeit that the medicine is formulated as a racemate), with three magnetically different methoxy functional groups, a *p*-disubstituted (methoxy) aromatic ring, and a 3,4-disubsituted (dimethoxy) veratrate ester aromatic ring, as well as displaying *o*- and *m*-coupling constants. Also, it has a methyl group adjacent to a (chiral) methine and a spectroscopically discrete *N*-ethyl functional group. The other five methylenes include a benzylic carbon (adjacent to the chiral center), and a short chain of

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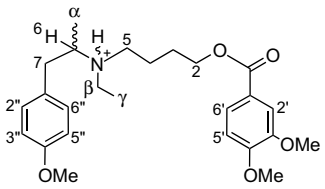
4×CH_2, one adjacent to *N*, another to *O*, and then two β- to *N* or *O* making for straightforward ^{1}H and ^{13}C NMR spectroscopic assignments (including teaching HMQC/HSQC, HETCOR or HMBC), at least in $CDCl_3$, and we thought this would be equally trivial in D_2O. From the ^{1}H NMR NOESY data in D_2O a possible cross-peak between two protons, one on each aromatic ring, had to be pursued to see if it was relevant to a potential bioactive conformation in aqueous media, which has not been previously reported. It was the subsequent dilution experiments which gave rise to the non-trivial results reported in this paper.



Surprisingly, there is only one literature report of NMR spectroscopic data for this drug,[25] and only one ^{1}H NMR spectroscopic study of its quantitative determination in $CDCl_3$ with the (six methylene) singlet of the tricyclic antibacterial methenamine (hexamine, $C_6H_{12}N_4$) as an internal reference standard.[26] In order to determine if our NOE cross-peak between the aromatic rings was a signal from an intramolecular interaction (or intermolecular or noise), we carried out dilution experiments. We observed that different non-exchangeable aromatic protons shift to different extents and can even result in coalescence of signals, thus significantly changing the appearance of the spectrum and its associated integration. This change in integration as a function of concentration can easily lead to misinterpretation of the spectroscopic data and even to controversy in the literature.[1] Such coalescence of signals leads to loss of connectivity in the ^{1}H–^{1}H COSY and ^{1}H–^{13}C HMQC spectra. Although solvent- and temperature-dependent chemical shift changes are well known,[27–29] and also the concentration-dependent shift changes of exchangeable hydrogens, the concentration-dependent chemical shift changes, which lead to ^{1}H NMR signal cross-over or coalescence of non-exchangeable hydrogens remain a rarity.[1,2] We propose a novel approach to obtain quantitative analytical data from the measurement of chemical shift migrations of selected aromatic protons on mebeverine HCl in the linear range. This method is a straightforward and highly reproducible.[1,2] It is amenable to the analysis of tablets either during manufacture or in a long-term stability testing study.

2. Results and discussion

2.1. ^{1}H NMR data

Supplementary data Figure 1 shows typical ^{1}H, ^{13}C, and HMBC NMR spectra for (*RS*)-mebeverine free base (150 mg/mL in $CDCl_3$) and the NMR spectra were assigned as detailed in Section 4.2.

Supplementary data Figure 2 shows typical ^{1}H and ^{13}C NMR spectra for mebeverine HCl (20 mg/mL in D_2O). These NMR spectra were assigned as detailed in Section 4.2.

From these two sets of assignments, of the free base and the HCl salt, we can see that in the ^{1}H NMR spectroscopic data the aromatic signals resonate in the same order, although at slightly different chemical shifts, but displaying similar coupling constants. The methoxy groups resonate in a different order, with 4′- and 3′-OMe coalesced in the free base, and the aliphatic signal order is significantly different, especially the shifts of benzylic H7 with its *geminal J* occurring up to 0.5 ppm apart. There are also some differences in the aromatic ^{13}C order. One key result, which could easily lead to disagreements in the literature, as elude to in the introductory paragraph,[1] is the relative change in integration values for the four groups of aromatic ^{1}H signals from 1:1:3:2 (0.1 mg/mL) to 1:1:2:3 (80 mg/mL). Here the pronounced migration of 5′ is easily seen (from 0.5 to 50 mg/mL, Fig. 1) although all the aromatic chemical shifts are shifting as a function of concentration in D_2O, but not in $CDCl_3$. At these intermediate concentrations (0.5 to 50 mg/mL, Fig. 1) there are five groups of aromatic ^{1}H signals integrating 1:1:2:1:2 in D_2O and in $CDCl_3$.

2.2. Nuclear Overhauser effect

Nuclear Overhauser effect (NOE) data for mebeverine HCl were collected in order to establish any preferred solution conformation. The cross-peaks in the resulting two-dimensional spectra connect resonances from protons that are spatially close (e.g., Supplementary data, Figs. 3 and 4). As there are no NOE studies for mebeverine HCl reported in the literature, one long-term aim is to measure (and ultimately to quantify) the conformation(s) of mebeverine in a range of solvents ($CDCl_3$, CD_3OD, and D_2O) and at various pHs (4, 7, 12, mebeverine pK_a=9.51±0.50)[25] and so to determine whether mebeverine is a long molecule or coiled around displaying π–π bond interactions, i.e., to answer the question 'are the two aromatic rings are close to each other'? From the NOE data (20 mg/mL), we see that there are cross-peaks between H5′ at 6.70 ppm of the benzoate ester and the –OCH_3 protons at 3.69 ppm, fixing –OCH_3 on C4′ of the benzoate ring. The cross-peak between H2′ at 7.16 ppm and –OCH_3 protons at 3.62 ppm fixes –OCH_3 on C3′ of the benzoate ring. There is only one –OCH_3 remaining (C4″ on the anisole ring), and a cross-peak between H3″,5″ of the anisole ring at 6.61 ppm and protons at 3.59 ppm (C4″ –OCH_3) settles this assignment unambiguously.

Also, there are cross-peaks between CH_3 (γ) on *N*-ethyl at δ 1.24 and CH_3 (α) on chiral C6 at δ 1.07, we conclude that in one conformation they are on the same side of the molecule. A cross-peak between CH_3 (α) at δ 1.07 and δ 3.69 –OCH_3 on C4′ of the benzoate ring is consistent with this conformation. However, on collecting NOE data with different concentrations, we concluded that other cross-peaks are due to interactions between atoms on different molecules (intermolecular) and not within the same drug molecule (intramolecular). Also, it was clear that the chemical shifts of the aromatic protons became four signals from five upon diluting the solution (from 20 to 0.1 mg/mL), so we therefore studied the ^{1}H NMR spectroscopic data from mebeverine HCl quantitatively to investigate this aromatic chemical shift migration.

2.3. Quantitative NMR study

The ^{1}H NMR spectra across a 4-orders of magnitude concentration range (0.01–100.0 mg/mL) of mebeverine HCl were collected in D_2O (Fig. 1). The results revealed a strong concentration-dependent chemical shift variation in ^{1}H NMR spectroscopic values. A novel methodology based on the proportionality of this dependence was then developed in the linear range (5–50 mg/mL) to achieve the quantitation of mebeverine HCl in aqueous solutions. The proposed method was evaluated regarding accuracy, precision, and robustness. The pH and temperature effects, as well as the applicability limitations of the method posed by current NMR instrumentation are discussed. NMR spectroscopic data were also recorded for mebeverine HCl in different solvents from D_2O, in four typical NMR solvents ($CDCl_3$, CD_3CN, DMSO-d_6, and CD_3OD) where concentration-dependent chemical shifts were not observed (Fig. 2).

Compared with the 0.25 ppm shift seen for aromatic signals, aromatic MeO signals migrated upfield by 0.15 ppm (from 20 to 100 mg/mL), and other aliphatic signals moved by <0.1 ppm. Therefore, the chemical shifts assigned to mebeverine aromatic protons were considered as the detector's response in order to

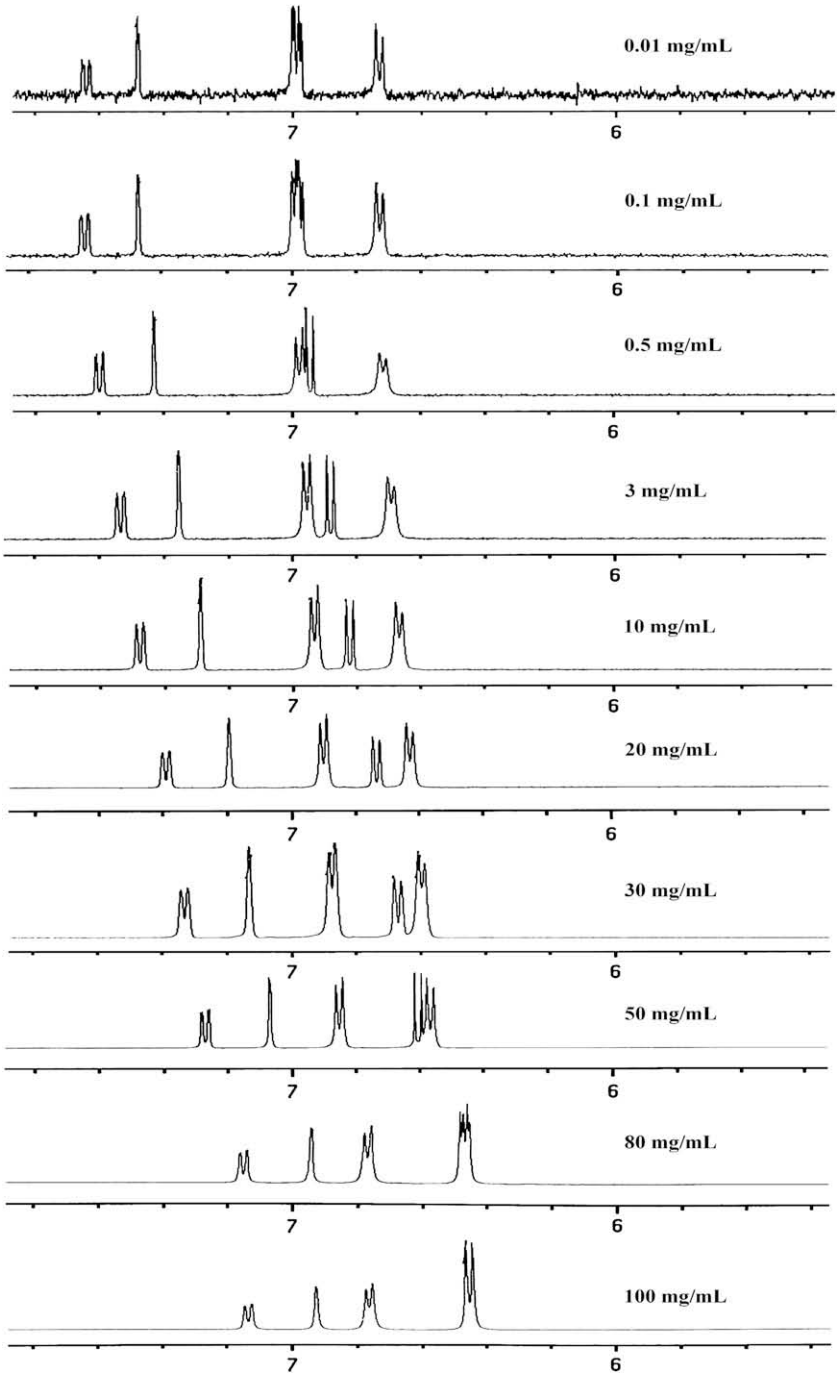


Figure 1. Partial ^{1}H NMR spectra of mebeverine HCl aromatic protons signals, at different concentrations ranging from 0.01 to 100 mg/mL recorded in D_2O.

develop a quantitative ^{1}H NMR methodology. Comparison of spectra obtained from samples of different concentrations showed that all proton resonances moved downfield upon dilution. These chemical shift displacements are more intense for the aromatic protons, and each displayed a linear range (Table 1). The responses from H3″,5″ (r^2=0.9998) were selected to derive the response function.

The plots of chemical shifts corresponding to mebeverine aromatic protons provided 5 calibration curves. As the results obtained from H3″,5″ signals were the optimum, this proton was selected for the calculations of mebeverine HCl concentration both in the authentic drug (Table 2) and in Colofac IBS tablets (Table 3). Applying the proposed NMR quantitative analytical method to the authentic drug resulted in excellent recovery as shown by a statistical comparison of the results obtained using Student's *t*-test and the Variance ratio test (*F*-test), which showed no significant difference between them (Table 2).[30]

2.4. Response function and linearity

The signal displacements induced by concentration changes follow a specific pattern. This pattern was further elucidated by investigation of spectroscopic data from calibration curve solutions. Chemical shift values (δ) of the aromatic protons were plotted against the concentration. A linear graph was obtained when these chemical shift values were correlated to the concentration (*C*). The

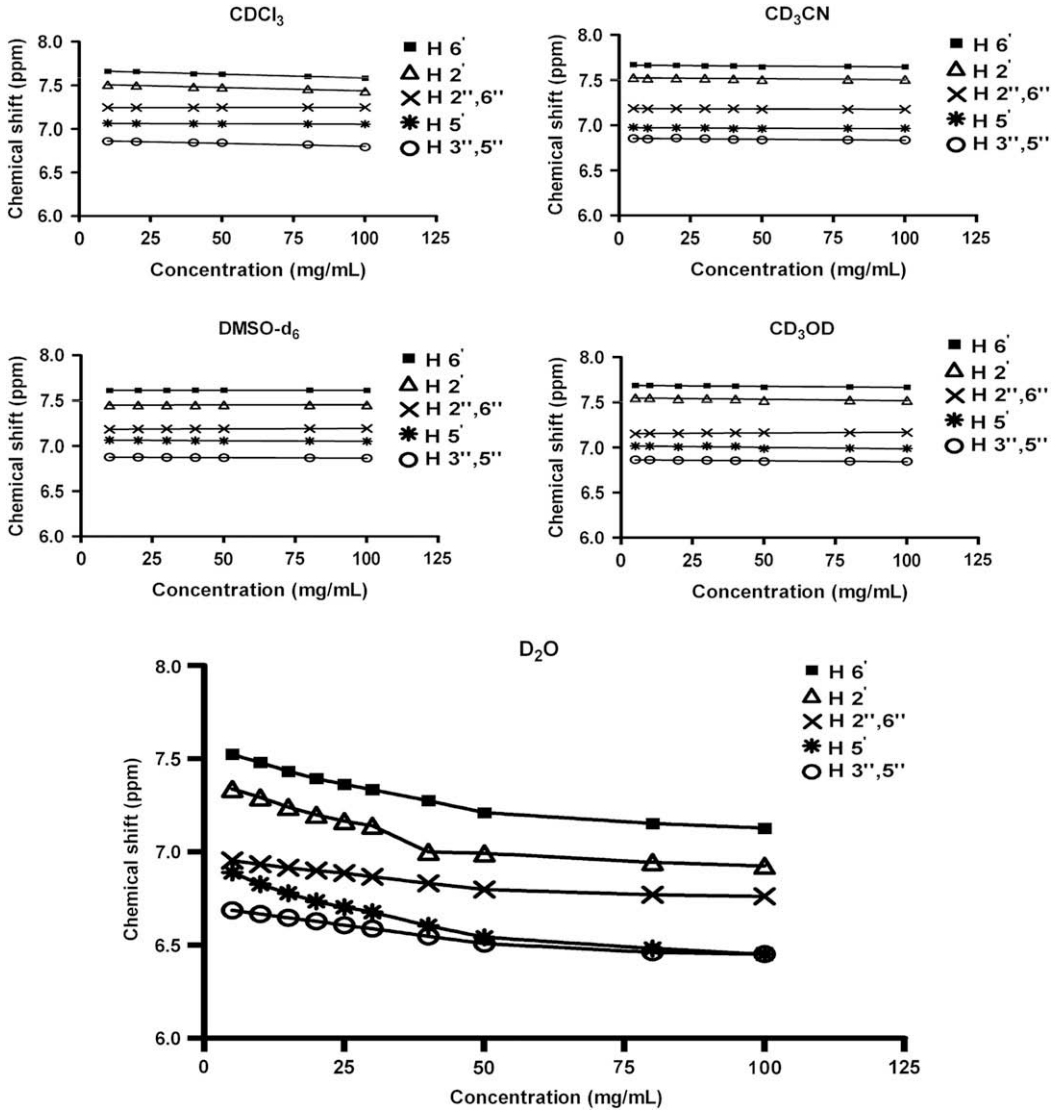


Figure 2. Concentration (mg/mL) versus chemical shift (ppm) of aromatic protons of mebeverine HCl in different solvents.

linear graph with the best regression equation was used for the calculations (linear graph of H3″,5″) and the linear range was found to be from 5 to 50 mg/mL.

2.5. Robustness

The experiments were repeated in solutions of 20 mg/mL with different acidic pD (pH measured in D_2O)[2,9] ranging from 1 to 5. The results obtained from all tested solutions were almost identical (Fig. 3). It is evident that chemical shift values are not influenced significantly within the selected pD range and therefore the method is applicable.

Table 1
Regression equations for various aromatic protons of mebeverine HCl in D_2O

Proton	Regression equation	r^2
2″,6″	δ=−0.0034C+6.9696	0.9984
3″,5″	δ=−0.0041C+6.7075	0.9998
2′	δ=−0.0075C+7.3625	0.9939
5′	δ=−0.0072C+6.8929	0.9925
6′	δ=−0.0068C+7.5425	0.9908

2.6. Temperature effect

Temperature control during the analysis is important because it can affect the degree of self-association phenomena and the reliability of the results. The change of chemical shift with

Table 2
Statistical analysis of the results obtained from the determination of mebeverine HCl by the proposed ^{1}H NMR spectroscopic method compared with the official B.P. method[23]

Concentration (mg/mL)	^{1}H NMR determined concentration (mg/mL)	Recovery %	^{1}H NMR method	Official B.P. method
5.0	4.96	99.2	Mean=99.33	Mean=99.83
10.0	9.86	98.6	SD±0.92	SD±0.51
15.0	15.1	100.7	V=0.85	V=0.26
20.0	19.6	98.0	t	1.12 (2.18)[a]
25.0	25.1	100.4	F	3.27 (3.97)[a]
30.0	29.6	98.7		n=6
40.0	39.9	99.8		
50.0	49.6	99.2		

[a] 2.18 is the theoretical t-value and 3.97 is the theoretical F-ratio at p=0.05, which our experimentally derived values should be below.[30]

Table 3
Statistical analysis of the results obtained from the determination of mebeverine HCl in Colofac IBS tablets by the proposed ^{1}H NMR method

Taken C (mg/mL)	Determined C (mg/mL)	Recovery %	
5.0	4.9	98.0	Mean=99.3
15.0	14.9	99.3	SD±0.92
20.0	19.9	99.5	V=0.84
25.0	24.7	98.8	
30.0	30.3	101.0	
40.0	39.5	98.8	
50.0	49.7	99.4	
13.5	13.5	100.0	Mean=97.7
13.5	13.3	98.5	SD±1.45
13.5	13.0	96.3	V=2.10
27.0	26.2	97.0	
27.0	26.0	96.3	
27.0	26.5	98.1	

r^2=0.9998 (for δ 6.61 ppm, H3″, H5″).

temperature was linear, showing an increase of 0.4 ppm from 25 to 75 °C (Fig. 4).

2.7. Quantitative analysis of Colofac IBS tablets

The results of quantitative analysis of Colofac IBS tablets (Table 3) from five crushed tablets, and taking weights equivalent to the given concentrations of pure drug (in 1 mL of D_2O), showed 99.3±0.92% recovery. Similarly, when 1 or 2 (135 mg) tablets were dissolved (in 10 mL of D_2O) 97.7±1.45% was the accuracy of the measured concentration (Table 3 and Supplementary data, Fig. 5). The Colofac IBS tablets are prepared (from the Patient Information Leaflet) containing sucrose and lactose. We calculated their approximate sugar excipient concentrations from the NMR spectroscopic data (Supplementary data, Fig. 5) to be sucrose (100±1 mg/135 mg tablet) and its $C_{12}H_{22}O_{11}$ isomer lactose (33±2 mg/135 mg tablet). In order to prove that this NMR shift effect is a function of the drug concentration alone, and not significantly affected by these excipients in the medicine formulation, we analyzed three concentrations of sugars with 27 mg/mL of mebeverine HCl in D_2O (1 mL), i.e., sucrose (100.3, 20.6, 4.2 mg, i.e., 5×, 1×, 0.2×) and lactose (35.6, 7.4, 1.6 mg), respectively. These excipients caused no change in the aromatic proton chemical shifts of mebeverine HCl.

2.8. Diffusion studies on mebeverine hydrochloride in D_2O, $CDCl_3$, and CD_3OD

One possible explanation for the observed concentration dependency of the mebeverine HCl aromatic ^{1}H resonances is the

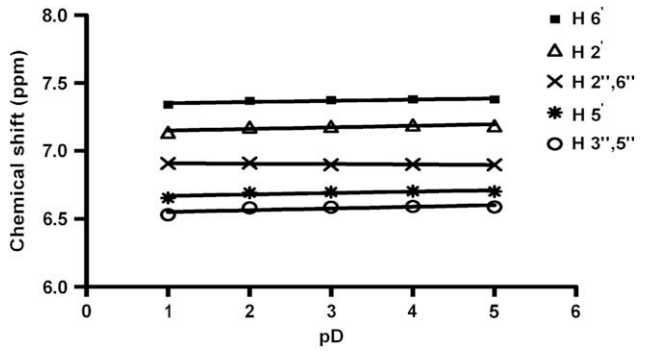


Figure 3. Effect of different pD (measured in D_2O with pH paper) on the chemical shift of the aromatic protons of mebeverine HCl.

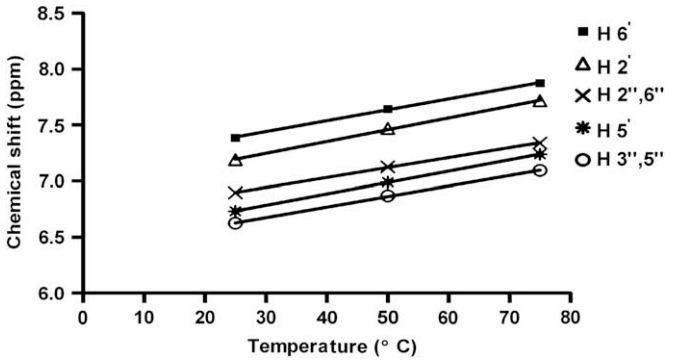


Figure 4. Effect of temperature on the chemical shift of the aromatic protons of mebeverine HCl.

formation of aggregates in solution, potentially via stacking interactions of the aromatic rings.[31] Pulsed gradient spin echo (PGSE)[32] experiments allow for the determination of the self-diff usion coefficient D_t. It is intuitive that D_t is related to the size of the diffusing species, and provided that the size of the molecules under consideration is substantially greater than the size of the solvent molecules then the Stokes–Einstein equation may reasonably be applied to gain information on the hydrodynamic radius (r_H)

$$D_t = \frac{kT}{6\pi\eta r_H}$$

where k is the Boltzmann constant, T is the temperature, and η is the solution viscosity.[33] However where the molecules of interest are considerably closer in size to the solvent molecules then a modified form of the Stokes–Einstein equation must be used:

$$D_t = \frac{kT}{c\pi\eta r_H}$$

In this case, c is a numerical factor that can be expressed as a function of r_H and the van der Waals radius of the solvent.[34]

$$c = \frac{6}{\left[1 + 0.695\left(\frac{r_{solv}}{r_H}\right)^{2.234}\right]}$$

It can also be shown that:

$$\frac{D_t^{sa}}{D_t^{so}} = \frac{c^{so} r_H^{so}}{c^{sa} r_H^{sa}}$$

where sa denotes sample and so solvent.[35] As D_t^{sa} and D_t^{so} can be experimentally determined and $c^{so}r_H^{so}$ can be calculated from known values, an estimate of $c^{sa}r_H^{sa}$ can be achieved; using a plot of $c^{sa}r_H^{sa}$ versus r_H^{sa}, r_H^{sa} may be found, and thus V_H.

Whereas the c factor is approximately 6 (5.81) in D_2O, it is closer to 4 in $CDCl_3$ and in CD_3OD (Table 4). The apparent hydrodynamic volume, V_H, for mebeverine HCl in D_2O solution is far greater (e.g., 10-fold) than that seen in either $CDCl_3$ or CD_3OD (Table 4), and this presumably reflects the aggregation of several molecules. An

Table 4
Diffusion coefficients D_t (10^{-10} m^2 s^{-1}), hydrodynamic radii r_H (Å), hydrodynamic volume V_H (Å^3), and c factors for mebeverine HCl solutions determined in $CDCl_3$, CD_3OD, and D_2O (all at 6 mg/mL)

Solvent	D_t	r_H	c	V_H
$CDCl_3$	14.3	3.73	4.53	217
CD_3OD	16.9	3.17	4.14	133
D_2O	3.02	6.80	5.81	1320

aggregation number, *N*, of approximately 6–7 can be estimated for this, though these approximate numbers are based upon a spherical molecule. For molecules that deviate from perfect spheres, the task of determining r_H is much harder and beyond the scope of this research. However, the difference in magnitude of the observed V_H is significantly large enough to suggest that the diffusion data confirm that the molecules do aggregate in aqueous solution.

3. Conclusions

Quantitative ^{1}H NMR spectroscopic analysis, via the use of either an external or an internal standard, has its drawbacks, e.g., line broadening (with a co-axially mounted capillary) may occur to such an extent that the small shifts we have observed due to solute–solute interactions cannot be measured with sufficient precision. Any added internal standard may also influence the weak solute–solute interactions. Chemical shift changes have been reported due to solute–solvent/solvent–solvent interactions,[36,37] host–guest complexation,[38,39] and the hydrophobicity of organic molecules.[40] From our study, we propose that the dramatic variation of the chemical shifts observed could be a result of molecular aggregation leading to the formation of differently packed assemblies. The non-covalent weak forces that stabilize assemblies are possibly intermolecular hydrogen bonds, π–π aromatic stacking, and electrostatic interactions, leading to 'head to head' and/or 'head to tail' dimers. In such assemblies, the number of molecules, the orientations in the aggregate, and their mutual interactions and 'tightness' of association should vary as a function of concentration which in turn should manifest in the altered chemical shifts.[1,2] A novel quantitative ^{1}H NMR spectroscopic method is proposed for mebeverine HCl analysis in aqueous solutions based on aromatic chemical shift migration as a function of concentration. This quantitative method displays acceptable characteristics regarding accuracy, precision, and robustness; it is not dependent on magnetic field frequency. The applicability of this method depends mainly on the extent of the association phenomena in aqueous solvents, but it is a robust method for the quantitative determination of mebeverine HCl by NMR chemical shift migration.

4. Experimental

4.1. Materials

Mebeverine HCl was kindly supplied by Solvay Healthcare Limited, UK, stored and protected from light. Colofac IBS tablets containing 135 mg of mebeverine HCl per tablet was purchased from Solvay Healthcare Limited, UK. Deuterium oxide (99.9% D) was purchased from Cambridge Isotope Laboratories, Inc. (USA), and deuterium chloride solution (99 at % D, 35 wt % in deuterium oxide, Sigma–Aldrich, Germany) was used for the preparation of acidic solutions.

4.2. ^{1}H NMR measurements

^{1}H NMR experiments were performed on Jeol Delta (270 MHz), Varian Mercury (400 MHz) and Varian Inova (600 MHz) NMR spectrometers. 1D spectra were acquired using 32K data points and zero-filled to 64K data points before Fourier transformation. Probe temperature was maintained at 25 °C. Chemical shifts are relative to the residual solvent peak.

Supplementary data Figure 1 shows typical ^{1}H, ^{13}C, and HMBC NMR spectra for (*RS*)-mebeverine free base (150 mg/mL in $CDCl_3$) and the NMR spectra were assigned to δ chemical shift in parts per million (integration, multiplicity, coupling constant absolute values in hertz): 7.63 (1H, dd, J=9, 2, H6′), 7.50 (1H, d, J=2, H2′), 7.04 (2H, d, J=9, H2″, H6″), 6.85 (1H, d, J=9, H5′), 6.77 (2H, d, J=9, H3″, H5″), 4.25 (2H, t, J=7, H2), 3.90 (6H, s, 3′-OMe and 4′-OMe), 3.74 (3H, s, 4″-OMe), 2.93–2.80 (1H, m, H6), 2.80 (1H, dd, J=13$_{gem}$, 4, H7), 2.56–2.43 (4H, m, H5 and Hβ), 2.32 (1H, dd, J=13$_{gem}$, 9, H7), 1.73–1.67 (2H, m, H3), 1.56–1.48 (2H, m, H4), 1.02 (3H, t, J=7, Hγ), 0.89 (3H, d, J=7, Hα). ^{13}C NMR (δ ppm, 150 mg/mL in $CDCl_3$): 166 C1, 158 C4″, 153 C4′, 149 C3′ (3×aromatic Cq-OMe), 133 C1″, 130 C2″ and C6″, 123 C6′, 123 C1′, 114 C3″and C5″, 112 C2′, 110.1 C5′, 65 C2, 57 C6, 56 C4′ and C4″ (2×methoxy groups), 55 C3′ (methoxy group), 49 C5, 44 Cβ, 39 C7, 27 C3, 26 C4, 14 Cγ and Cα. Assignments were made using HSQC, NOE, and HMBC spectra.

Supplementary data Figure 2 shows typical ^{1}H and ^{13}C NMR spectra for mebeverine HCl (20 mg/mL in D_2O). These NMR spectra were assigned to δ chemical shift in parts per million (integration, multiplicity, coupling constant absolute values in hertz): 7.36 (1H, dd, J=9, 2, H6′), 7.16 (1H, d, J=2, H2′), 6.89 (2H, d, J=9, H2″, H6″), 6.70 (1H, d, J=9, H5′), 6.61 (2H, d, J=9, H3″, H5″), 4.26–4.17 (2H, non-first order m, H2), 3.69 (3H, s, 4′-OMe), 3.62 (3H, s, 3′-OMe), 3.59 (3H, s, 4″-OMe), 3.53–3.50 (1H, m, H6), 3.18–3.08 (4H, m, H5 and Hβ), 2.84 (1H, dd, J=14, 4, H7), 2.54 (1H, dd, J=14$_{gem}$, 10, H7), 1.79–1.73 (4H, m, H3 and H4), 1.24 (3H, t, J=7, Hγ), 1.07 (3H, d, J=7, Hα). ^{13}C NMR (δ ppm, in D_2O): 168 C1, 158 C4′, 153 C3′, 148 C4″ (3×aromatic Cq-OMe), 130 C2″, C6″, 128q C1″, 124 C6′, 122q C1′, 114 C3″, C5″, 112 C2′, 111 C5′, 65 C2, 60 C6, 55.8 (4′), 55.5 (3′), 55.3 (4″) (3×Ar methoxy groups), 50 C5, 46 Cβ, 36 C7, 25 C3, 22 C4, 13 Cγ, 10 Cα. Assignments were made using HSQC and NOE spectra. Not unexpectedly, there were no chemical shift changes with spectrometer field strength (270, 400, and 600 MHz).

4.3. Preparation of solutions

A stock standard solution of mebeverine HCl (100 mg/mL) in deuterium oxide was used to prepare the calibration standard solutions. At least 10 different concentrations ranging from 0.01 to 100 mg/mL were used for the calibration of the chemical shift scale. Aliquots of 1 mL from each calibration solution were transferred to 5 mm NMR tubes and each ^{1}H NMR spectrum was recorded in triplicate. For the evaluation of method robustness, samples in D_2O with pD 1, 2, 3, 4, 5 (against pH paper)[2,9] were prepared by addition of appropriate amounts of 0.01 M DCl in aliquots of 20 mg/mL of mebeverine HCl solution. The effect of temperature was further evaluated by recording the ^{1}H NMR spectra of 20 mg/mL of mebeverine HCl standard solutions at 25, 50, and 75 °C.

Five tablets of Colofac IBS tablets were accurately weighed and ground to a fine powder. Amounts of the tablet powder equivalent to 5.0–50.0 mg of mebeverine HCl were transferred to small vials and dissolved in D_2O (1.0 mL), filtered through a nylon membrane (0.45 μm), and then transferred to a 5 mm NMR tube. Additionally, 1 or 2 Colofac IBS tablets (135 or 270 mg) were dissolved in D_2O (10.0 mL, n=3) and analyzed quantitatively by ^{1}H NMR spectroscopy.

4.4. PGSE measurements

All PGSE measurements were determined using a Varian Mercury 400 MHz spectrometer equipped with a 4 nucleus auto-switchable probe, using the Dbppste pulse sequence, at 25 °C, without spinning the sample. Gradient strength (G) was varied over 10 spectra, which were acquired with 32K data points, over a spectral width of 5 MHz, with a relaxation delay of 5 s and processed with line broadening of 0.3 Hz. In each case the diffusion of residual protic solvent (e.g., $CHCl_3$ in $CDCl_3$) was used as an internal standard since the diffusion rates are known.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2009.02.026.

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