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A simple method for the identification and assay of iopamidol and iothalamate meglumine in pharmaceutical samples based on proton nuclear magnetic resonance spectroscopy

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A proton nuclear magnetic resonance (PMR) spectroscopic method is described for the direct assay and identification of the triiodinated radiographic contrast agents iopamidol (nonionic type) and iothalamate meglumine (ionic type) in commercial solutions and as a bulk material. Samples were prepared by simply diluting an injectable solution with or dissolving a powdered sample in D_2O. Sodium acetate was added to serve as an internal standard. Quantitations were based on the resonance signals for the protons of the CH_3-CO-group at 1.58 ppm (iopamidol) or 2.25 ppm (iothalamate), CH_3-N-group at 2.38 ppm (meglumine) and CH_3-CO-group at 1.92 ppm (acetate). The mean $\pm$SD (n = sets of 10 samples each) recovery of iopamidol, iothalamic acid and meglumine from synthetic mixtures with the internal standard were 99.6 ± 0.63, 99.7 ± 0.66 and $99.9 \pm 1.18\%$, respectively; with the values ranging from 98.7–100.9% for iopamidol and iothalamate, and from 98.3–100.8% for meglumine.

1. Introduction

Triiodinated radiographic media for parenteral use comprise a group of water-soluble derivatives of benzoic acid which, upon intravascular administration, are capable of opacifying the vasculature of the organs they perfuse and the organ systems that are responsible for their excretion [1, 2]. Hence, these drugs are of clinical utility in the radiographic visualization of blood vessels, the urinary and hepatobiliary tracts, and certain parts of the spinal cord and brain [2, 3].

At present, the most commonly used triiodinated radiographic agents are the monomers of either a nonionic compound containing iodine atoms to particles in a ratio of 3 : 1 [4], or of a salt that in solution dissociates into a triiodinated benzoate anion and an accompanying organic or inorganic counterion and yields an iodine atoms to ions in a ratio of 3 : 2 [4]. Among monomeric forms, certain nonionic compounds (e.g., iopamidol) and ion-paired combinations (e.g., iothalamate meglumine) are found advantageous since their solutions exhibit a lower hyperosmolality and, hence, a lower tendency to render the plasma severely hypertonic [4, 5]. Plasma hypertonicity is of clinical concern because it is a frequent cause for osmotoxic effects such as vascular pain, hypotension, neural irritation, and even neural and vascular damage [1–5].

The quantitative determination of iodinated radiographic contrast media in pharmaceutical samples has been, for the most part, indirect and relying on the measurement of the iodine content [6–15]. These methods are valid since the free iodine present in commercial iodinated radiographic solutions is usually undetectable [13]; but their execution requires equipment that is either rather elaborate or specialized or a methodology that is both lengthy and complicated [6, 7]. Indeed, in most instances a multistep dehalogenation process is needed to convert the organically-bound iodine to an inorganic form amenable to titrimetric analysis [5–14]. Direct analytical approaches, including direct titration of the carboxylic acid functionality [11, 12] and polarography [16], have also been proposed but they are few in number. Furthermore, a direct nondestructive technique such as HPLC has been applied [17–19] whenever substitution at the carboxyl group renders the aromatic moiety resistant to dehalogenation [6]. On the other hand, the aminosugar meglumine has been measured separately by polarimetry [20], titrimetry [21, 22] and HPLC [23] when present as a paired ion.

The purpose of the present report is to describe a PMR spectroscopic for the analysis of both ionic (meglumine iothalamate) and nonionic (iopamidol) triiodinated radiographic agents. This method is extremely simple, shows good accuracy, and will be able to concurrently measure meglumine and its accompanying cationic counterion. In addition it will permit the unambiguous identification of the various analytes when present singly or ion-paired.

2. Investigations, results and discussion

The feasibility of quantitatively analyzing iodinated benzoic acid derivatives for radiographic use by PMR spectroscopy was previously demonstrated in this laboratory with the ion-paired combinations diatrizoate meglumine and diatrizoate meglumine plus diatrizoate sodium [24]. In the present study, this analytical approach has been extended to include the nonionic compound iopamidol and the ionizable salt iothalamate meglumine. Owing to their good solubility in water, analytical samples of these radiographic agents were readily prepared by either dissolving the bulk material in or diluting the commercial injectable solution with D_2O. In contrast, solubilization of iothalamic acid in this solvent necessitated the addition of a trace of sodium hydroxide solution to form the more soluble sodium salt. Sodium acetate was found to be appropriate as an internal standard for all of the samples tested since its methyl protons resonated as a sharp and well resolved signal.

The PMR spectra of each of the compounds and salts examined in the present study are shown in Figs. 1–4. Table 1 lists the chemical shifts, multiplicities and resonance assignments for the protons of the internal standard and the radiographic agents. From these data it is evident that the proposed PMR spectroscopic method can be used for the identification of the various radiographic agents. The merits of this application are further realized, for instance, when trying to differentiate between triiodinated benzoic acid derivatives diatrizoic acid and iothalamic acid, two functional isomers whose only structural difference is the

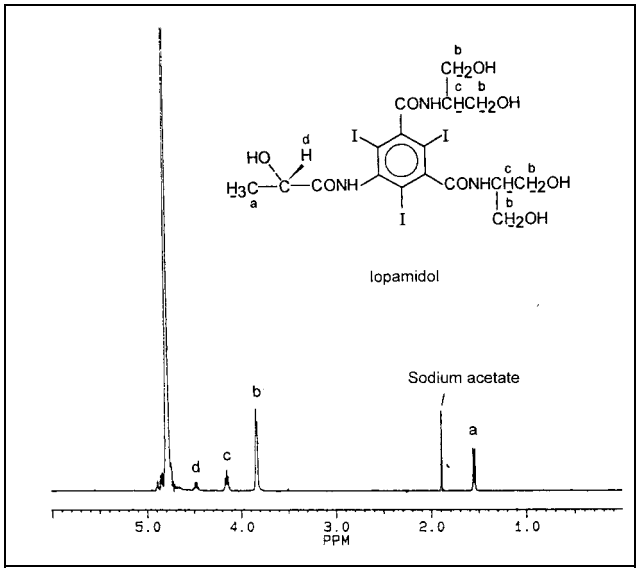

Fig. 1: PMR spectrum of iopamidol in D_2O

Table 1: PMR spectroscopic data for iopamidol, iothalamate, meglumine and sodium acetate in D_2O

Chemical shift (ppm)	Number of protons	Multiplicity	Assignment	EW*
Iopamidol				
1.58	3	doublet	$CH_3-CH(OD)-$	259.03
3.86	8	doublet	$-CH_2(OD)$	
4.18	2	multiplet	$-ND-CH-$	
4.51	1	multiplet	$CH_3-CH(OD)-$	
Iothalamate				
2.25	3	singlet	CH_3-CO-	204.64
2.95	3	singlet	CH_3-ND-	
Meglumine				
2.38	3	singlet	CH_3-ND-	65.07
2.61–2.75	2	multiplet	$-CH_2-ND-$	
3.59–3.67	2	multiplet	$-CH_2(OD)$	
3.75–3.92	4	multiplet	$>CH(OD)$	
Sodium acetate				
1.92**	3	singlet	CH_3-CO-	27.34

* EW is the formula mass of the compound divided by the number of protons contributing to the resonance selected for quantitation
** This signal was shifted to 2.03 ppm in the presence of iothalamic acid

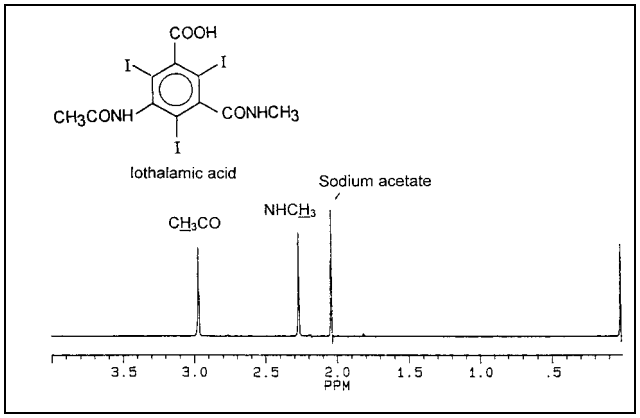

Fig. 2: PMR spectrum of iothalamic acid and sodium acetate, the internal standard, in D_2O

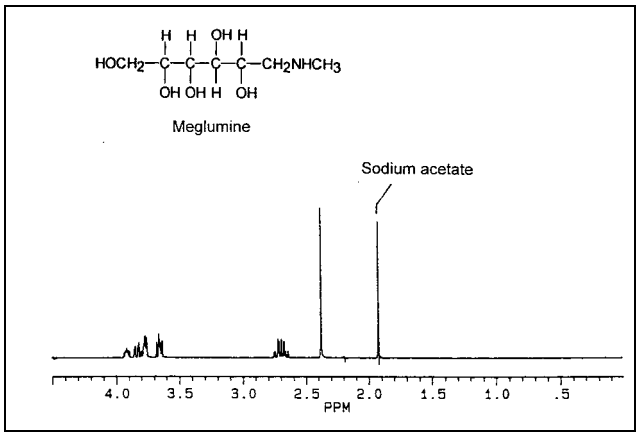

Fig. 3: PMR spectrum of meglumine and sodium acetate, the internal standard, in D_2O

substitutent at C-5 of the aromatic ring. Thus, whereas in diatrizoic acid C-5 bears an acetylamino group (resonating at 2.25 ppm) [24], in iothalamic acid this position is occupied by an *N*-methylcarbamyl group (resonating at 2.95 ppm).

The accuracy of the PMR spectroscopic method was tested by analyzing a set of 10 synthetic mixtures containing the internal standard with iopamidol or with iothalamic plus meglumine in the amounts listed in Tables 2 and 3. These results indicated that the accuracy of the proposed method was not affected by the range of relative proportions of analyte to internal standard examined.

Several lots of commercial samples of iopamidol and of iothalamate megluminate solutions for injection were also analyzed by the PMR spectroscopic method. The results of this study are summarized in Tables 4 and 5. Since this method represents a direct approach to measuring iodinated radiographic materials, it will yield results in terms of the percent weight of the compound or ion-paired salt combination per volume unit (i.e., g/100 ml), rather than as the percent weight of iodine present per volume unit (i.e., mg iodine/100 ml) which is the case with indirect methods. The % w/v values reported in Table 5 were calculated based on the amount of iothalamic acid found and the declared amount of iothalamic acid in the sample of iothalamate meglumine tested. Alternatively, the same results could be obtained from the sum of the amounts of iothalamate and meglumine found (Table 5) [24]. The drug content of all of the samples tested agreed closely

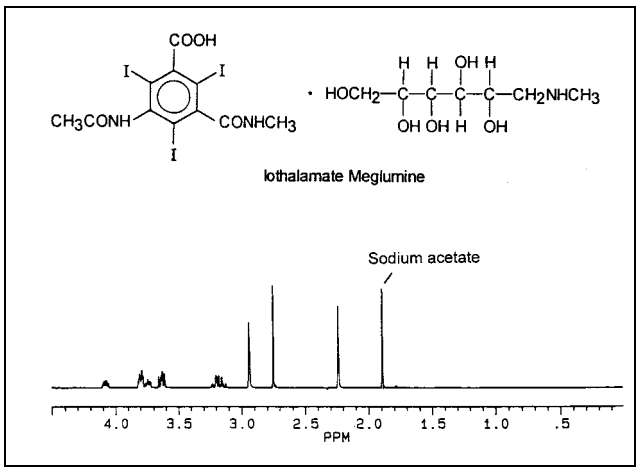

Fig. 4: PMR spectrum of iothalamate meglumine and sodium acetate, the internal standard, in D_2O

Table 2: Recovery of iopamidol from synthetic samples by PMR spectroscopy

Sample number	Sodium acetate added (mg)	Iopamidol		
		Added (mg)	Found (mg)	Recovery (%)
1	13.0	123.1	121.9	99.9
2	11.3	107.0	107.6	100.6
3	11.8	111.7	110.9	99.3
4	11.2	106.0	105.5	99.5
5	10.8	102.3	101.8	99.5
6	10.5	99.4	99.9	100.5
7	12.8	121.2	120.5	99.4
8	10.3	97.5	97.3	99.8
9	10.9	103.2	103.5	100.3
10	12.3	116.5	115.0	98.7
Mean				99.8
SD				0.63

Table 3: Recovery of iothalamic acid and meglumine from synthetic mixtures by PMR spectroscopy

Sample number	Sodium acetate added (mg)	Iothalamic acid			Meglumine		
		Added (mg)	Found (mg)	Recovery (%)	Added (mg)	Found (mg)	Recovery
1	10.1	103.8	102.9	99.1	25.0	24.9	99.6
2	11.2	127.9	129.0	100.9	24.3	24.5	100.8
3	12.3	151.2	150.7	99.7	25.1	24.8	98.8
4	10.5	81.9	81.2	99.1	24.9	25.1	100.8
5	12.8	112.3	111.5	99.3	24.6	24.9	101.2
6	11.2	149.5	150.2	100.5	24.2	23.8	98.3
7	13.2	105.7	104.9	99.2	25.3	24.9	98.4
8	10.9	128.9	128.5	99.7	23.9	24.2	101.3
9	10.5	101.6	100.9	99.3	25.4	25.2	99.2
10	13.8	126.5	127.1	100.5	24.8	25.0	100.8
Mean				99.7			99.9
SD				0.66			1.18

with the declared drug contents and were well within the limits of acceptance specified in USP 23 [19, 20].

In summary, the PMR spectroscopic method described here will greatly simplify the identification and quantitative analysis of both ionic and nonionic iodinated radiographic agents in pharmaceutical samples. Additional advantages of this method are its ability to concurrently measure meglumine and not to require the use of pure analytes as standards.

Table 4: Assay results for commercial solutions of iopamidol by PMR spectroscopy

Sample number	Found (mg/ml)	Found (% of declared)*
61% Solution		
1	605.3	99.2
2	602.2	98.7
3	615.1	100.8
4	608.4	99.7
5	604.3	99.1
Mean		99.5
Range		98.7–100.8
76% Solution		
1	750.2	98.7
2	745.3	98.1
3	758.2	99.7
4	748.1	98.4
5	749.2	98.6
Mean		98.7
Range		98.1–99.7

* USP 23 requirements are not less than 95.0 percent and not more than 105.0 percent of the labeled amount of iopamidol

Table 5: Assay results for iothalamate meglumine commercial solutions by PMR spectroscopy

Sample number	Iothalamic acid Found (mg/ml)	Meglumine Found (mg/ml)	Iothalamate meglumine*	
			Found (mg/ml)**	Found (% of declared)***
60% Solution – Lot A				
1	460.5	143.4	603.9	100.1 (100.7)
2	458.3	142.8	601.1	99.6 (100.2)
3	450.2	141.9	592.1	97.6 (98.7)
4	452.7	142.7	595.4	98.4 (99.2)
5	451.8	141.5	593.3	98.2 (98.9)
Mean				98.8 (99.5)
Range				97.6–100.1 (98.7–100.7)
60% Solution – Lot B				
1	453.9	144.3	598.2	98.7 (99.7)
2	457.3	145.2	602.5	99.9 (100.4)
3	454.3	143.8	598.1	98.8 (99.7)
4	453.2	142.9	596.1	98.5 (99.4)
5	452.5	143.3	595.8	98.4 (99.3)
Mean				98.9 (99.7)
Range				98.4–99.9 (99.3–100.4)

* The USP 23 requirement is not less than 95.0 per cent and not more than 105.0 percent of the labeled amount of iothalamate meglumine
** Based on the content of iothalamic acid found
*** Based on the content of iothalamic acid plus meglumine found

3. Experimental

3.1. Apparatus

All PMR spectra were obtained with a Model AM-400 spectrometer (Bruker Instruments, Billerica, MA, USA), operating at a temperature of 28 °C and using the following conditions: data point resolution, 0.125 Hz/point; relaxation delay, 10 s; number of scans, 32; and window function, none.

3.2. Materials

The samples of iopamidol and iothalamate meglumine were USP reference standards (U.S. Pharmacopeial Convention, Inc., Rockville, MD, USA). Iothalamic acid was a gift from the manufacturer (Mallinckrodt Diagnostics, St. Louis, MO, USA). Meglumine was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Deuterium dioxide (D_2O, 99.8 atom% D), 3-(trimethylsilyl)propionic acid sodium (TSP, 99.9 + %) and sodium acetate (99.995%) were obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI, USA). Samples of iopamidol USP injection 61% and 76% and of iothalamate meglumine USP 60% were purchased from local commercial sources.

3.3. Assay method

An aliquot (0.5 ml) of injectable solution, an accurately weighed quantity of sodium acetate (30–40 mg), and sufficient D_2O to bring the volume to 2 ml were successively placed in a graduated, glass-stoppered, centrifuge tube. After stoppering, the mixture was mixed with the aid of vortexing to effect solution, and a portion of the liquid (about 0.1 ml) was transferred to an analytical NMR tube that contained a crystal of TSP. After diluting with D_2O (about 0.55 ml) and stoppering, the contents of the tube were mixed by inversion, and the resulting solution was used to obtain the PMR spectrum. All chemical shifts were referenced with respect to TSP, taken as 0.00 ppm on the δ scale. The signals for the internal standard (sodium acetate, 1.90 ppm) and analyte (iopamidol, 1.58 ppm; iothalamate, 2.25 ppm; meglumine, 2.38 ppm) were integrated and used to calculate the quantities of triiodinated radiographic contrast agent and meglumine in each milliliter of injectable solution tested according to the following equations:

$$\text{Iopamidol or iothalamatic acid (mg/ml)} = (A_b/A_s) \times (EW_s/EW_b) \times (S/V)$$

$$\text{Meglumine (mg/ml)} = (A_m/A_s) \times (EW_s/EW_m) \times (S/V)$$

were A_b, A_s and A_m are the average integral values for the resonances of the triiodinated benzoic acid derivative (iopamidol or iothalamic acid), the internal standard and meglumine, respectively; EW_s, EW_b and EW_m are

the formula weight equivalents, i.e., formula weight/number of absorbing protons, for the triiodinated benzoic acid derivative (777.09/3 = 259.03 for iopamidol; 613.92/6 = 102.32 for iothalamic acid), the internal standard (82.03/3 = 27.34) and meglumine (195.21/3 = 65.07), respectively; S is the quantity of internal standard taken for the analysis, mg; and V is the volume of injectable solution taken for the analysis, ml.
Alternatively, the percentage of ionizable radiographic contrast agent in the sample tested was calculated from the quantities of iothalamic acid and meglumine found and either one of the following equations:

$$\text{Iothalamate meglumine (\%)} = 100 \times \frac{\text{Iothalamic acid found (mg/ml)} + \text{meglumine found (mg/ml)}}{\text{Amount of iothalamate meglumine declared (mg/m)}}$$

$$\text{Iothalamate meglumine (\%)} = 100 \times \frac{\text{Iothalamic acid found (mg/ml)}}{\text{Amount of iothalamate meglumine declared (mg/m)}}$$

where the quantities of iothalamate declared, mg/ml = 455.24 for a 60% solution, based on a 1 : 1 molar stoichiometry between the triiodobenzoate moiety and the megluminium moiety.

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