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Short communication

A novel quantification method of pantaprazole sodium monohydrate in sesquihydrate by thermogravimetric analyzer☆

V. Ranga Reddy [a], M. Anantha Rajmohan [a], R. Laxmi Shilpa [a], Dilip M. Raut [a], Kolla Naveenkumar [b], M.V. Suryanarayana [a,*], Vijayavitthal T. Mathad [b]

[a] *Department of Analytical Research and Development, IPD, Dr. Reddy's Laboratories Ltd., Unit-III, Plot No. 116, S.V. Co-Op. Ind. Estate, Bollaram, Jinnaram, Medak Dist 502325, Andhra Pradesh, India*

[b] *Department of Research and Development, IPD, Dr. Reddy's Laboratories Ltd., Unit-III, Plot No. 116, S.V. Co-Op. Ind. Estate, Bollaram, Jinnaram, Medak Dist 502325, Andhra Pradesh, India*

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Abstract

To demonstrate the applicability of thermogravimetric analyzer as a tool for the quantification of pantaprazole sodium monohydrate in sesquihydrate, studies have been conducted. Thermal analysis (DSC, TGA) crystallographic (PXRD) and spectroscopic techniques (FT-IR) were used for the characterization of the polymorphs. Thermogravimetric analysis (TGA) analysis was explored by high-resolution dynamic (Hi-Res-dynamic) and high-resolution modulated (Hi-Res-modulated) test procedures to quantify the hydrate polymorphic mixtures. The two polymorphic forms exhibited significant differences and good resolution in the second derivative thermogram generated by Hi-Res-modulated test procedure. Thus, the TGA with Hi-Res-modulated test procedure was considered for the quantification of monohydrate in sesquihydrate. The calibration plot was constructed from the known mixtures of two polymorphs by plotting the peak area of the second derivative thermogram against the weight percent of monohydrate. Using this novel approach, 1 wt% limit of detection (LOD) was achieved. The polymorphic purity results, obtained by TGA in Hi-Res-modulated test procedure were found to be in good agreement with the results predicted by FT-IR and was comparable with the actual values of the known polymorphic mixtures. The Hi-Res-modulated TGA technique is very simple and easy to perform the analysis.

Keywords: Thermogravimetric analysis; Hi-Res-dynamic; Hi-Res-modulated; Pantaprazole sodium; Monohydrate; Sesquihydrate; Quantification; Polymorphism

1. Introduction

The ability of an organic molecule to exist in more than one crystalline form exhibit different physico-chemical properties like density, solubility, dissolution rate and bioavailability. Additional complexities can also arise due to the difference in flow properties, compact ability, crystal morphology and hygroscopicity [1,2]. The pseudo-polymorphic forms exhibit stoichiometric and non-stoichiometric compositions of solvate or hydrate present in the crystal lattice, which results in different physico-chemical and mechanical properties from the true polymorphic forms. Hydrates may be stable within a wide range of relative humidity (RH). The non-stoichiometric forms of solvated/hydrated forms with different degrees of crystallinity, results in a stable or unstable crystal structures [3]. Polymorphism and its variation in the degree of crystallinity in a pharmaceutical substance may exhibit physicochemical differences that impacts therapeutic, manufacturing, commercial, and legal levels [4–8]. The abrupt change in the crystalline form during the manufacturing or storage can threaten process development, which may lead to serious consequences [9,10]. Therefore, quantification of polymorphic forms due to stringent quality control measures of these different solid forms in active pharmaceutical ingredients (API) and drug product is a challenging task for analytical chemists associated with pharmaceutical business. The pharmaceutical companies engaged in generic products try to generate all the polymorphic forms of an API and aims to characterize those missed by the innovator [11,12]. At the time of the abbreviated new drug application (ANDA) submissions,

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* Corresponding author. Tel.: +91 8458 279572; fax: +91 8458 279619.
E-mail address: suryamv@drreddys.com (M.V. Suryanarayana).

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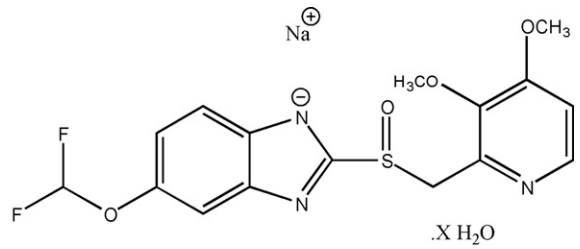

Where, if X=1.0 Monohydrate

X=1.5 Sesquihydrate

Fig. 1. Chemical structure of pantaprazole Na.

the potential release of the new drug product would depend on the legal situation and one cannot compromise on the detection limits or the resolution of the equipment. The quantification of the reported polymorphic forms in the novel polymorphic form with a good limit of detection (LOD) is a major concern. However, the United States Food and Drug Administration (USFDA) allows the use of alternative forms provided, the criteria of pharmaceutical equivalence and Bio equivalence are met [13].

Several techniques are currently used for the study of quantification of undesired polymorphic forms in the desired one. Although, X-ray diffraction remains the most common techniques for identifying and characterizing polymorphs [14], vibrational spectroscopic analysis (FTIR, NIR, Raman) has also become popular as a complimentary technique to monitor crystallinity and polymorphism due to its speed, minimal sample preparation, and adaptability for online use [15]. The advantages of Thermal Analysis and vibrational spectroscopic methods are, the ease of sample preparation and minimal sample requirement (approximately 10 mg) to perform the analysis.

For this study, pantaprazole Na (monohydrate and sesquihydrate), sodium salt of 5-(difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridinyl) methyl] sulfinyl]-1H-benzimidazole (Fig. 1) that inhibits gastric acid secretion was selected to demonstrate the applicability of TGA as a tool for quantification of hydrate polymorphic forms. The Hi-Res-modulated TGA technique was validated for reproducibility and precision of pantaprazole Na monohydrate in sesquihydrate. The quantitative analysis of polymorphic mixtures of pantaprazole Na by Hi-Res-modulated TGA was compared with the FT-IR analysis to test the predictive power of Hi-Res-modulated TGA.

2. Experimental

2.1. Materials

Pantaprazole sodium monohydrate was synthesized as per the process described in the patent [16]. The sesquihydrate was synthesized by dissolving the pantaprazole free base [17] (50 g, 0.130 mol) in a mixture of sodium hydroxide (5.4 g, 0.134 mol) (The Andhra Sugars Ltd., Tanuku, India), de-ionized water (10 mL) and 350 mL of tetra hydro furan (THF) (TCC chemicals, Taiwan), followed by precipitation of salt by addition of 400 mL dichloromethane (Standard Laboratories, Hyderabad, India) as an anti-solvent to the resulting mixture at 25–35 °C. Finally, it was cooled to 0–5 °C and the precipitated solid was filtered to obtain the sesquihydrate as a fine solid material. The polymorphic purity was confirmed by PXRD, DSC and FT-IR.

2.2. Preparation of standard polymorphic mixtures

The known quantities of pure polymorphic forms of pantaprazole Na monohydrate and sesquihydrate were weighed separately on a Mettler Toledo micro analytical weighing balance with an accuracy of 0.01 mg. The components were ground gently to obtain the homogeneity. The total weight of the samples was kept constant (100 mg) for each set of spiked sample preparation. Several set of spiked samples of concentrations 2, 3, 5, 10, and 15 wt% of monohydrate in sesquihydrate were prepared for the calibration plot of method development. The test samples of polymorphic ratios 6 and 13 wt% of monohydrate were also prepared for the comparison study.

2.3. Powder X-ray diffraction (PXRD)

The powder X-ray diffraction studies were performed by filling the sample holder by top loading method and exposing the sample to CuKα radiations (45 kV × 35 mA) in a wide-angle X-ray Diffractometer of BRUKER axs, D8 ADVANCE (GmbH, Karlsruhe, West Germany). The instrument was operated in the continuous scan mode in increments 0.02° (2θ). The angular range was 3–45° (2θ) and counts were accumulated for 0.4 s at each step. Data acquisition and analysis were performed on BRUKER Diffrac plus EVA software.

2.4. Differential scanning calorimeter (DSC)

The analyses were performed on DSC Q1000 (TA Instruments, New Castle, Delaware, USA) under the nitrogen gas purge at a flow of 50 mL/min. The instrument was calibrated for temperature and heat flow using indium as standards. The samples were encapsulated into closed aluminum pans with pinhole and subsequently crimped to ensure a tight seal. Data acquisition and analysis were performed using Universal Analysis 2000 software (TA Instruments).

2.5. Thermogravimetric analysis (TGA)

The analyses were performed on TGA Q500 of TA Instruments (Lukens Drive, Delaware, USA), in Hi-Res (High Resolution) mode with dynamic and modulated test procedure under the nitrogen gas purge at a flow of 40 mL/min for balance and 60 mL/min for sample. Instrument was calibrated for temperature with Nickel (supplied by the TA instruments) and TGA balance was calibrated with certified weights (Denver Instrument-calibration weights, Denver, Colorado). The experimental conditions for dynamic test procedure, in which the samples were analyzed on the platinum pan were, with a heating rate of 20 °C/min up to 190 °C and resolution number of +4 with sensitivity value of 1. The experimental conditions for

the modulated test procedure were with modulation temperature amplitude of ±5 °C, modulation period of 200 s, resolution number of 6 with sensitivity value 1 and a heating rate of 5 °C/min up to 160 °C. Data acquisition and analysis were performed using Universal Analysis 2000 software (TA Instruments).

2.6. Fourier transform infrared spectroscopy (FT-IR)

FT-IR analyses were performed on Spectrum One FTIR spectrometer (Perkin-Elmer, USA) with 45 number of scans and resolution of 4 cm^{-1} in the scan range of 450–4000 cm^{-1}. The instrument was calibrated by using Polystyrene film. Sample preparation was carried out by mixing 5–10 mg of a solid in a smooth agate mortar with 1–2 drops of the paraffin liquid heavy (NICE chemicals Pvt. Ltd., Cochin, India) (Mull technique) thoroughly. Spectral treatment was performed using the Perkin-Elmer spectrum software version of 5.0.1.

3. Results and discussion

3.1. Analysis of pantaprazole Na monohydrate and sesquihydrate by powder X-ray diffraction (PXRD) and differential scanning calorimetry (DSC)

Pantaprazole Na monohydrate and sesquihydrate were characterized by PXRD. Fig. 2 shows significant difference in the diffraction patterns of Pantaprazole Na hydrates. The sesquihydrate contains all the diffraction peaks of monohydrate in addition to its significant characteristic peaks. Therefore, non-interfering peak of monohydrate was not observed to establish the calibration plot for the quantification of monohydrate in sesquihydrate.

Fig. 3 shows the DSC thermograms of pantaprazole Na monohydrate and sesquihydrate obtained at a heating rate of 10 °C/min. The dehydration endothermic peak of Pantaprazole Na sesquihydrate and monohydrate are at 143.69 and 154.14 °C, respectively. This shows the difference in the dehydration temperature of pantaprazole Na monohydrate and sesquihydrate. Due to overlapping of monohydrate melting peak with the decomposition peak of sesquihydrate, correct estimation of the area under the peak was not possible, hence the quantification method was not developed. The use of Chemometrics method, such as PLS (Partial Least Square) to measure the calibration standards using PXRD & DSC would be helpful and effective in the above case.

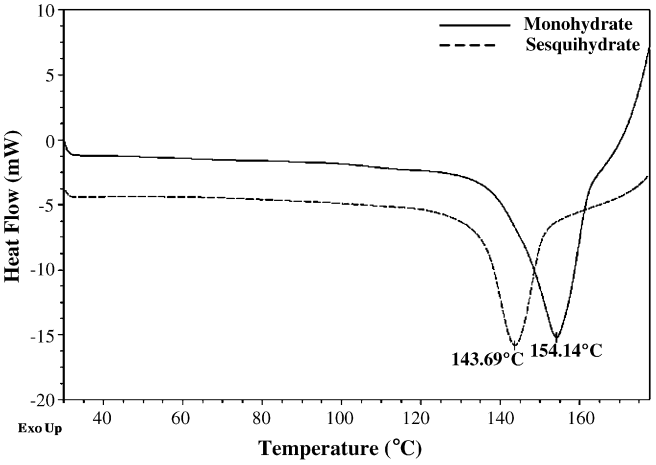

Fig. 3. Overlaid DSC thermograms of pantaprazole Na monohydrate and sesquihydrate.

3.2. Analysis of pantaprazole Na monohydrate in sesquihydrate by thermogravimetric analyzer (TGA)

TGA experiments were performed in Hi-Res (High Resolution) mode with dynamic and modulated test procedure. The heating rate of TGA in dynamic test procedure was similar to that of the conventional, except for the actual heating rate, which varies dynamically during the experiment in response to the rate of weight change (%/min). As the percent of weight change increases, heating rate decreases and vice-versa.

TGA thermograms of Hi-Res-dynamic test procedure were obtained for pantaprazole Na monohydrate and sesquihydrate to identify the differences between the two polymorphic forms. Fig. 4 shows the second derivative thermogram of two polymorphs. It is observed that the second derivative thermogram exhibits separation between two polymorphs.

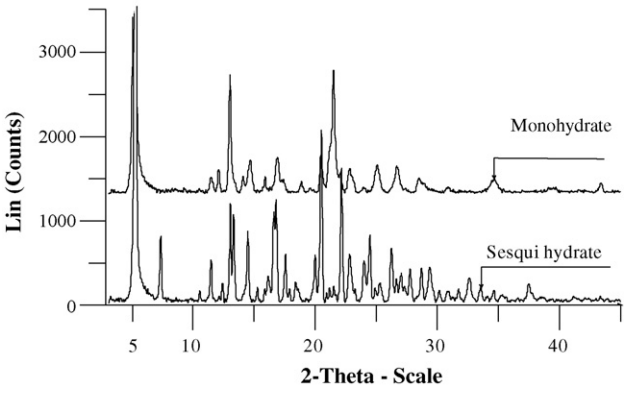

Fig. 2. Overlaid diffraction patterns of pantaprazole Na monohydrate and sesquihydrate.

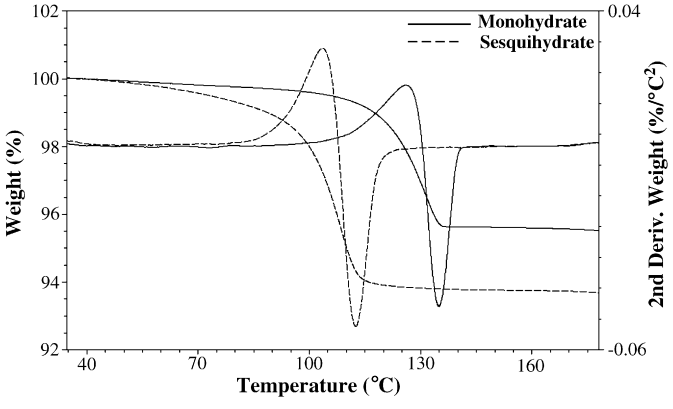

Fig. 4. Hi-Res-dynamic TGA thermograms of pantaprazole Na monohydrate and sesquihydrate with respect to second derivative.

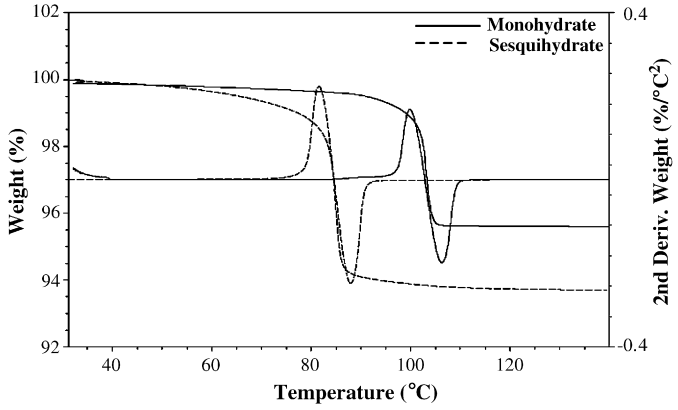

Fig. 5. Hi-Res-modulated TGA thermograms of pantaprazole Na monohydrate and sesquihydrate with respect to second derivative.

Table 1
Accuracy and reproducibility of Hi-Res-modulated TGA in quantitative measurement of pantaprazole Na monohydrate in sesquihydrate

Actual wt% (spiked)	Calculated wt%	%Recovery
5	4.9	98
	5.1	102
	5.3 (%RSD = 3.92)	106
10	9.8	98
	10.3	103
	10.5 (%RSD = 3.53)	105
15	15.3	102
	14.4	96
	15.5 (%RSD = 3.8)	103

The TGA analysis in Hi-Res-modulated test procedure provides the same information as the conventional, plus the additional information about the behavior of the weight loss reaction. The sinusoidal heating profile applied to the material in modulated TGA, depends on underlying heating rate, amplitude of modulation and period of modulation. Fig. 5 shows the thermograms obtained for Hi-Res-modulated test procedure. The second derivative thermogram generated in the modulated test procedure exhibits good resolution between the two polymorphic forms than that of the thermograms generated in the dynamic test procedure. This can be owed to the modulation period and amplitude during the sinusoidal heating profile. Thus, the method for the quantification of monohydrate in sesquihydrate was developed by modulated test procedure. To investigate the potential of modulated TGA technique for quantitative polymorphic analysis, several calibration standards containing 2, 3, 5, 10, and 15 wt% of monohydrate in sesquihydrate were analyzed.

The weight loss (%) observed in the TGA thermogram was calculated by converting the thermogram into the second derivative. The peak area of second derivative thermogram was integrated linearly by using Universal Analysis 2000 software (TA Instruments) to obtain the area of monohydrate with respect to the weight loss. The areas obtained for different concentrations 2, 3, 5, 10, and 15 wt% of monohydrate in sesquihydrate, were found to increase with the increasing concentration of monohydrate. The limit of detection (LOD) was determined by signal to noise ratio at 1.0 wt% of monohydrate in sesquihydrate and the calibration plot was found to be linear with a regression coefficient (r^2) of 0.995.

To check accuracy and precision of the method, the spiked concentrations of 5, 10, and 20 wt% of monohydrate in sesquihydrate were analyzed in triplicate by Hi-Res-modulated TGA test procedure and the results are tabulated in Table 1 along with the mean and percent relative standard deviation (% RSD). It was observed that the calculated wt% (monohydrate) values predicted by Hi-Res-modulated TGA is in good agreement with the actual wt%. The low %RSD indicates that the method is precise and reproducible. This clearly infers that Hi-Res-modulated TGA test procedure is good for the determination of polymorphic composition of hydrates.

3.3. Comparing the Hi-Res-modulated TGA and FT-IR

To validate the Hi-Res-modulated TGA method, a FT-IR method was developed for pantaprazole Na monohydrate in sesquihydrate. The FT-IR spectrum was obtained for the two polymorphs to observe the differences between them. Fig. 6a illustrates the FT-IR spectrum of two polymorphs in the range of 450–4000 cm^{-1}. It was observed that both forms have distinguished characteristic bands, which can be used for their polymorphic identification.

In the region of 748–723 cm^{-1} monohydrate shows a significant band at 736 cm^{-1} with respect to the sesquihydrate as shown in Fig. 6b. The selection of a reference band at 1492 cm^{-1}, which is common for both polymorphic forms, acts as an internal standard. This would minimize the matrix effects caused by the sample preparation and crystal form composition. A set of calibration standards of 3, 5, 10 and 15 wt% of monohydrate in sesquihydrate were used to build the calibration graph. The areas under (736 cm^{-1}) peak corresponding to monohydrate (undesired polymorph) and (1492 cm^{-1}) reference peak were calculated by selecting the peak area option in the software (Perkin-Elmer spectrum software version of 5.0.1). The area was integrated with 2-point base line type, where a baseline was drawn by connecting the two points of the peak base-1 and base-2. The area under the monohydrate peak was found to increase with the increasing concentration. The limit of detection (LOD) for this method was 2 wt% of monohydrate in sesquihydrate and the calibration plot was found to be linear with a regression coefficient (r^2) of 0.991.

The predictive power of FT-IR method was tested by analyzing several test mixtures of pantaprazole Na sesquihydrate containing the known amount of monohydrate and comparing the results predicted from the linear calibration graph with the actual weight percent values. The results obtained from the linear calibration graph along with mean value of three-repeated scan analysis are shown in Table 2. It is observed that the predicted values are in close agreement with the actual weight percent values, indicating that the linear calibration graph is accurate for the quantification of monohydrate in sesquihydrate.

For the comparative studies of the results obtained by Hi-Res-modulated TGA and FT-IR, two samples of different concentrations, i.e., 6 and 13 wt% of monohydrate were

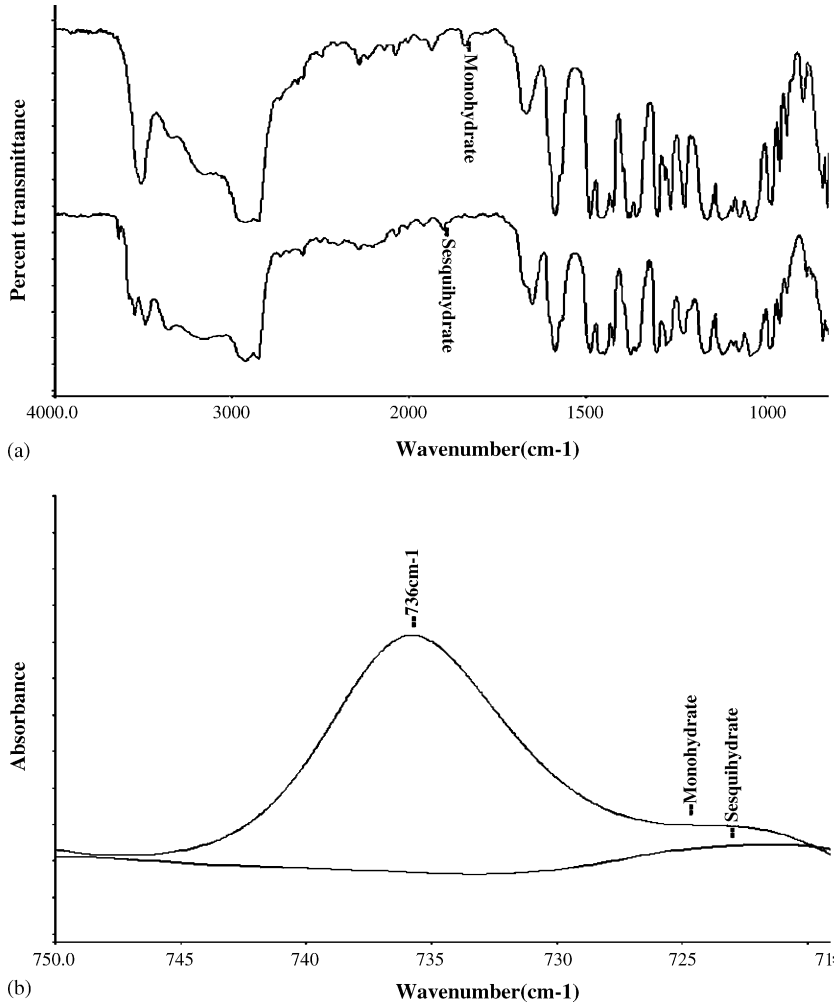

Fig. 6. (a) Stacked FT-IR spectra of pantaprazole Na monohydrate and sesquihydrate. (b) Zoomed FT-IR absorption spectra of pantaprazole Na monohydrate and sesquihydrate.

analyzed and their respective calibration graphs were used to determine the polymorphic purity in mixtures. The monohydrate weight percentage results thus obtained, using two techniques, are given in Table 3. The results obtained by two techniques are comparable to each other and in good agreement with the actual weight percent values.

In comparison to FT-IR, Hi-Res-modulated TGA technique is very simple and easy. No sample preparation is involved in the latter, where the spiked sample is directly taken into the pan to perform the analysis. This aids in obtaining accurate weight percent values and low LOD level, when compared to FT-IR technique.

Table 2
Accuracy and reproducibility of FT-IR in quantitative measurement of pantaprazole Na monohydrate in sesquihydrate

Actual wt% (spiked)	Predicted wt% by FT-IR	% Recovery
5	5.2	104
	4.8	96
	5.1 (% RSD = 4.13)	102
10	9.5	95
	10.1	101
	10.5 (%RSD = 5.0)	105
15	15.2	101
	14.4	96
	15.8 (%RSD = 4.6)	105

Table 3
Comparison of results obtained by Hi-Res-modulated TGA and FT-IR for polymorphic mixtures of pantaprazole Na monohydrate in sesquihydrate

Actual wt% (spiked)	Predicted wt% by Hi-Res-modulated TGA	Predicted wt% by FT-IR
6.0	5.96	5.83
	6.02	6.39
	6.23	6.12
13.0	13.21	12.98
	13.08	12.85
	13.15	13.02

4. Conclusions

In this work, we have reported a method to quantify hydrates of Pantaprazole Na (monohydrate in sesquihydrate) by Hi-Res-modulated TGA test procedure. The performance

of Hi-Res-modulated TGA for quantitative analysis was comparable with that of a well-established technique of FT-IR in terms of precision, reproducibility and sensitivity. The limit of detection (LOD) for Hi-Res- modulated TGA and FT-IR were 1.0% and 2.0%, respectively. The results obtained in this study clearly demonstrates the potential of Hi-Res-modulated TGA, which proves to be relatively simpler and better technique for the quantification of hydrate polymorphic forms.

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