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Characterization of polymorphs of a new anti-inflammatory drug

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Abstract: The present study demonstrates the utility of a diversified analytical approach for the characterization and quantitative analysis for two polymorphs of a new anti-inflammatory agent, (±)-7-[3-(4-acetyl-3-methoxy-2-propylphenoxy)propoxy-3,4-dihydro-8-propyl-2H-1-benzopyran-2-carboxylic acid (SC-41930). The existence of two distinct crystal polymorphs of SC-41930 was qualitatively indicated through microscopy and application of thermal methods of analysis. The application of TGA was important for establishing that the two solid forms were, in fact, polymorphs, as opposed to solvated and unsolvated drug substances. The application of IR spectrometry revealed spectral features in the carbonyl stretching region, which were characteristic and unique to the two SC-41930 polymorphs. DRIFT spectrometry was implemented as the sampling method of choice to eliminate the possibility of polymorphic transformations during conventional mulling or KBr pellet sampling procedures. The DRIFT spectrometry procedure permitted development of a quantitative assay for detection of the low-melting polymorph (as an impurity) in high-melting samples. Calibration plots showed acceptable linearity of response from 0 to 25% (w/w) low-melting samples spiked into the high-melting polymorph. The performance characteristics of the method indicated good run-to-run and day-to-day consistency for its intended use.

Keywords: *Polymorphism in pharmaceuticals; drug substance analysis; DRIFT spectrometry.*

Introduction

Crystal polymorphism has been recognized as an important element of drug development and manufacture for a number of years [1, 2]. The crystalline morphology of a drug substance is a determinant factor for physical parameters such as solubility, surface tension, density, crystal shape, chemical stability and bioavailability. The drug development process often involves selection of the polymorph that is the most efficacious as well as incorporation of control of the crystalline morphology into the synthesis and formulation processes. Previous studies have demonstrated that different crystalline polymorphs and amorphous forms of drugs such as novobiocin, sulfameter, and chloramphenicol, exhibit different responses to biological systems and underscore the importance of selection of the most effective polymorph for development [3–5]. A recent report by Kawashima *et al.* described preparation of new crystalline forms of tranilast, an antiallergic agent with low water solubility and bioavailability [6]. The new polymorphs of tranilast had improved availability *in vitro*, relative to previously characterized crystalline forms. Additional studies have characterized polymorphic transformations of drug substances due to a variety of factors, including solvent-mediated transformations [7] and subjecting the sample to thermal and mechanical energy or tabletting procedures [8–11]. (The references cited above are representative of an extensive field.)

An important part of studies that concern drug substance polymorphism is the battery of physical analytical methods that are used to characterize the samples. A requirement for such studies is to establish whether or not samples with different melting ranges are crystal polymorphs, solvates, or polymorphs of solvates. An additional requirement, which pertains specifically to the development and manufacture of drug substances, is that the methods should provide sensitive qualitative and quantitative polymorph determinations. Historically, a number of methods have been prevalent for studying polymorphism and polymorphic transformations in drug substances, including, X-ray crystallography, thermal analysis, dissolution, microscopy, dialometry, IR spectrometry and NMR [1]. The present study uses a combination of physical analytical

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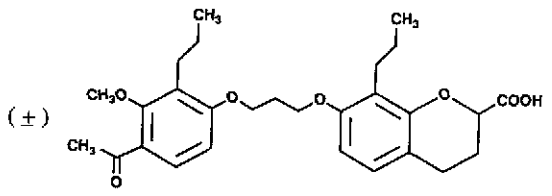

Figure 1
Structure of SC-41930.

methods, including differential scanning calorimetry (DSC), thermal gravimetric analysis (TGA), microscopy, and IR spectrometry to study two polymorphs of a new anti-inflammatory drug, (±)-7-[3-(4-acetyl-3-methoxy-2-propylphenoxy)propoxy-3,4-dihydro-8-propyl-2H-1-benzopyran-2-carboxylic acid (SC-41930), which has recently undergone clinical trials. The structure of SC-41930 is shown in Fig. 1. Microscopy, DSC and TGA have been used to distinguish low-melting (LM) and high-melting (HM) samples of SC-41930, and to provide evidence that the samples are crystalline polymorphs. The study has also emphasized the development of a diffuse reflectance infrared Fourier transform (DRIFT) spectrometry method, which uses mid-IR wavelengths, for sensitive qualitative and quantitative polymorph determinations for SC-41930 lots. A major advantage resulting from the use of DRIFT spectrometry is that the sample preparation circumvents potential problems associated with polymorph determinations performed with other solid state sampling techniques. The preparation procedure required for DRIFT spectrometry experiments does not subject the sample to thermal or mechanical energy sufficient to induce polymorphic transformations.

Experimental

Apparatus

Thermal analyses were completed with a DuPont 9900 Thermal Analyzer equipped with 912 DSC module and a 951 TGA Module. Polarized, transmitted light microscopy was performed with a Zeiss Universal Microscope, with camera. Samples were prepared for infrared spectrometry experiments by mixing with a Wig-L-Bug Amalgamator (Crescent Dental Mfg. Co.). Infrared spectra were obtained using a Nicolet 710 FT–IR Spectrometer equipped with a DTGS detector and a diffuse reflectance cell (The Collector™, Spectra-Tech, Inc.), and by implementing Nicolet 'Advantage' software (v2.14). The instrument and the diffuse reflectance cell were separately aligned, prior to sample analysis, in order to achieve the maximum energy throughput.

Materials and reagents

Low-melting (LM) and high-melting (HM) samples of SC-41930 were provided by the Chemical Development Department of Searle (Stokie, IL, USA). Samples were dried for 72 h at 50°C prior to milling and then passed through a 16 mesh screen. Samples utilized for the present study had chemical purity in excess of 99%, as determined by HPLC and DSC. Specific rotation and chiral HPLC data indicated that SC-41930 samples were racemic. The weight per cent composition of the capsule formulation was as follows: SC-41930, 48.78; lactose monohydrate NF, 48.78; hydroxypropyl methylcellulose USP, 1.95; sodium lauryl sulphate NF, 0.49. Spectroscopic-grade KCl and KBr were purchased from Aldrich Chemical Company and dried at 105°C prior to use.

Procedures

Thermal methods. Parameters for DSC were as follows: sample weight, 3 mg; sample cell, open pan; nitrogen pressure maintained at 30 PSI; heating rate, 10°C min^{-1}. Parameters for TGA were as follows: sample weight, 5 mg; sample cell, open pan; nitrogen pressure maintained at 30 PSI; heating rate, 20°C min^{-1}.

Microscopy. Slides for microscopy were prepared by dispersing a small amount of sample in mineral oil (refractive index — 1.47) between a glass slide and a cover slip. The slides were viewed in polarized, transmitted light with a magnification of 150–1000×. Photomicrographs were taken with Polaroid 559 colour film at magnifications of 160 and 400×.

Transmission IR spectrometry. Pellets of the SC-41930 for transmission IR experiments were 0.5% (w/w) in approximately 300 mg of KBr. The pellets were formed by applying 8–9 metric tons of pressure in a laboratory press for 1 min, unless designated otherwise. A normal scanning mode was employed for all samples and the background determinations, which included the following instrument (optical bench) settings: resolution, 4 cm^{-1}; aperture, 'large'; mirror velocity, 'normal'; gain, 1.

DRIFT spectrometry. Samples were prepared for DRIFT spectrometry by dispersing the drug substance (polymorphs) in KCl that had been previously ground in a Wig-L-Bug mixer for 2 min. Standards and samples were mixed with the Wig-L-Bug mixer for 30 s. Exclusion of the pestle in the Wig-L-Bug vial provided a homogenous sample, without subjecting the sample to thermal and mechanical stress. Typically, 400 mg of dispersant–sample mixtures were prepared, which contained 5% (w/w) of the drug substance. For calibration studies, low-melting samples (LM) were spiked into high-melting samples (HM). Mixtures of the LM and HM samples were shaken in the Wig-L-Bug mixer for 30 s prior to being mixed with the dispersant. Samples and standards were each placed into a 13 mm macro sample cup (which was part of the diffuse reflectance cell) and levelled with a spatula. After sample cup placement into the diffuse reflectance cell, the instrument sample housing compartment was purged with nitrogen for at least 3 min to diminish the presence of water and carbon dioxide vapours. Instrumental settings for DRIFT experiments were the same as for the transmission IR experiments, except the mirror velocity, which was 'slow' (5 kHz). Final DRIFT spectra resulted from subtracting the KCl spectrum from the sample spectra. Peak areas for quantitative measurements were determined by integration with the Advantage software.

Results and Discussion

Physical characterization of SC-41930 polymorphs

Physical characterization early in the development process for SC-41930 provided initial evidence for the possibile existence of SC-41930 polymorphs. It was found that early lots of SC-41930 chemical had two distinct melting ranges, 69–71 and 80–82°C. Table 1 summarizes USP melting range and bulk density data for three representative LM and HM samples. The bulk density for the HM samples was found to be two to four times greater than the bulk density for LM samples. Crystal polymorphism was considered the probable basis for the two forms, especially since the molecular structure of SC-41930 precluded the possibility of *cis/trans* isomerism or tautomerism as a basis for the melting range differences. Experimental results from microscopy, thermal analysis, and IR spectrometry experiments further distinguished the LM and HM forms and collectively provided evidence that the forms were crystal polymorphs of SC-41930. Microscopy revealed that the two forms had different crystal habits. The photomicrographs in Fig. 2 show that the LM samples [Fig. 2(A)] were thin needles while the HM samples [Fig. 2(B)] were platelets. Also, the difference in crystal habits for the two forms was considered the basis for the difference in bulk density. Representative DSC scans for the LM and HM forms shown in Fig. 3(A) and (B) provided preliminary indication that samples were not mixtures of different forms. Evidence that the samples were not solvates of SC-41930 was provided from the results in Fig. 4(A) and (B), which are TGA traces for LM and HM samples. Desolvation due to loss of a solvent-adduct prior to decomposition at 350°C was not observed.

Table 1
Melting range and bulk density data for LM and HM samples

Sample	Melting range (°C)	Bulk density (g cc^{-1})
LM	69.0–70.5	0.12
LM	70.0–71.5	0.11
LM	69.5–70.1	0.07
HM	80.0–82.0	0.24
HM	80.0–82.0	0.26
HM	79.6–82.3	0.30

Preliminary characterization of the SC-41930 polymorphs was also conducted with IR spectrometry since IR spectral features of polymorphs are often different, due to different molecular arrangements in the crystal lattice. Initial experimentation with transmission IR techniques revealed that differences existed in the carbonyl stretching frequency region of the spectra for HM and LM samples. The carbonyl stretching frequency region (1500–1800 cm^{-1}) of the spectra for the HM and LM samples are shown in Fig. 5(A) and (B), respectively, and show that absorbance bands at 1740, 1710 and 1670 cm^{-1} are unique to the LM form of SC-41930. The absorbances at 1645 and 1760 cm^{-1} were more prominent in the HM form.

Since the desired form for the large-scale synthesis of SC-41930 was the HM form, it was necessary to develop a sensitive polymorph test that was amenable to routine use. The intent of the test procedure was to document that lots

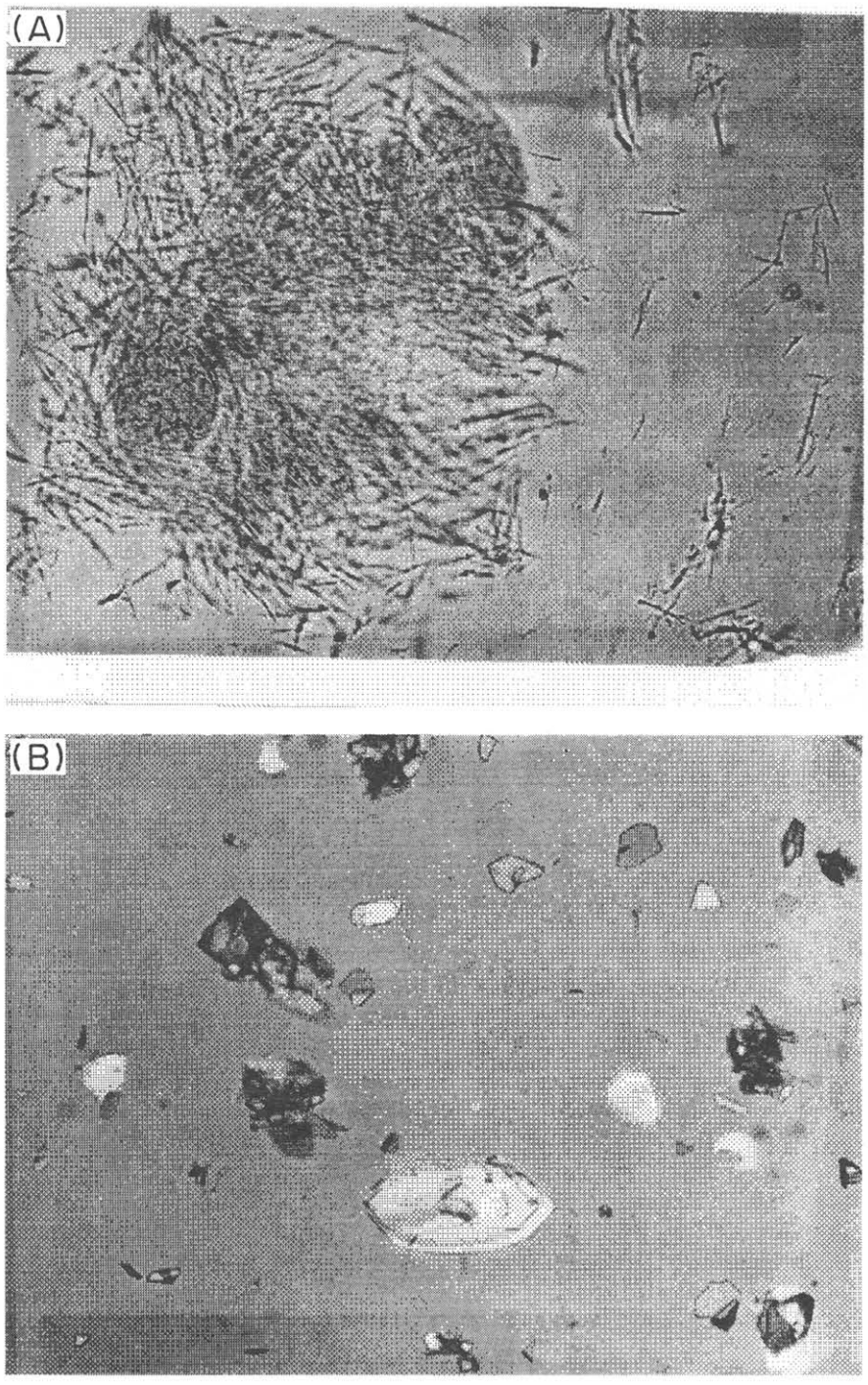

Figure 2
Photomicrographs of SC-41930 samples: (A) LM (400×), (B) HM (160×).

synthesized at Searle were the designated HM polymorph and not a mixture of LM and HM forms. The test would be used in tandem with DSC melting range or other thermal analysis data for confirmation of sample polymorph identity. Features of transmission IR spectra unique to the LM polymorph [Fig. 5(B)] were considered to be a basis for a qualitative and quantitative method; however, it was found that a key spectral feature was dependent on the KBr pellet formation procedure. Spectra in Fig. 5(A) and (B) were obtained by subjecting the samples dispersed in KBr to 8–9 metric tons for 1 min. When the samples were

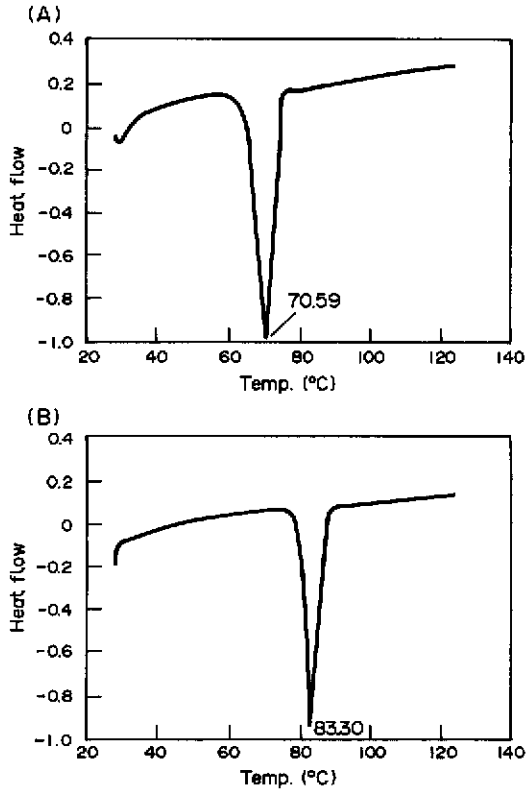

Figure 3
DSC scans of SC-41930 samples: (A) LM, (B) HM.

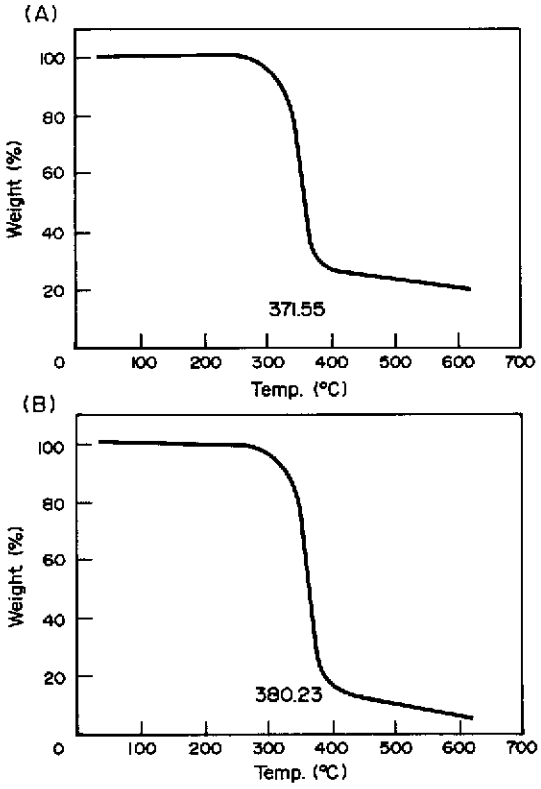

Figure 4
TGA scans of SC-41930 samples: (A) LM, (B) HM.

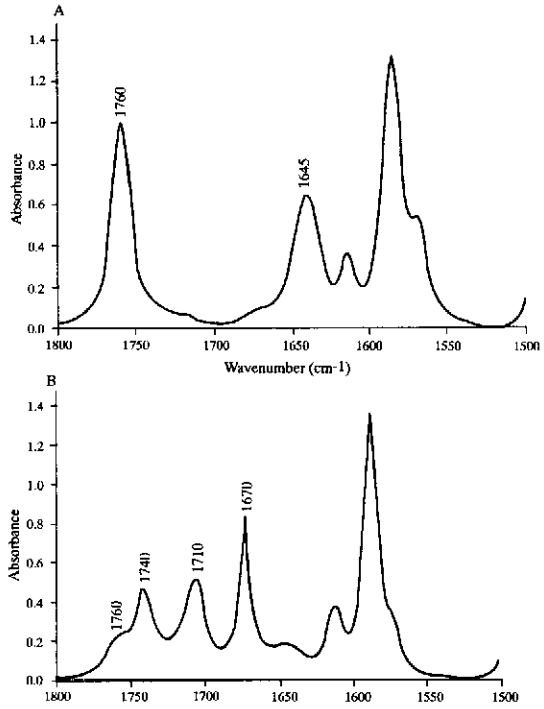

Figure 5
Expanded carbonyl region transmission IR spectra for SC-41930 samples: (A) HM sample, (B) LM sample.

subjected to pellet formation pressure for an additional 2 min, it was found that the procedure change resulted in a change in the bandwidth of the 'shoulder' absorbance at 1760 cm^{-1} for LM samples. Similar changes in the spectra of LM samples were observed after the mortar and pestle grinding required as part of preparation of a mineral oil mull for the LM form. Ibrahim *et al.* [12] and Takahashi *et al.* [13] have previously noted that solid sample preparation procedures for transmission IR spectra can induce changes in the spectra of polymorphs.

DRIFT spectrometry for polymorph determinations

Since it was found that the sample preparation procedures for the transmission IR approaches caused changes in the IR spectra in the LM samples that could be attributed to polymorphic transformations, DRIFT spectrometry was evaluated as a possible analytical method. DRIFT spectrometry was originally developed by Griffiths *et al.* [14] and has previously been applied to drug analysis [15, 16]. Since DRIFT spectra are obtained for powdered samples dispersed in a non-absorb-

ing inorganic salt such as KCl, the sample is not subjected to extensive pressure or grinding prior to analysis. Various forms of reflectance spectroscopy have been utilized previously for characterization of drug substance polymorphs. Ibrahim *et al.* [12] and Takahashi *et al.* [13] used attenuated total reflectance IR for distinguishing polymorphs of phenylbutazone and fostedil, respectively. Gimet and Luong utilized near-IR diffuse reflectance in conjunction with multiple linear regression analysis to quantitate polymorph levels of a new drug substance in a solid dosage form [17].

Expanded carbonyl region DRIFT spectra of HM and LM samples (5% w/w in KCl) are shown in Fig. 6(A) and (B), respectively. The

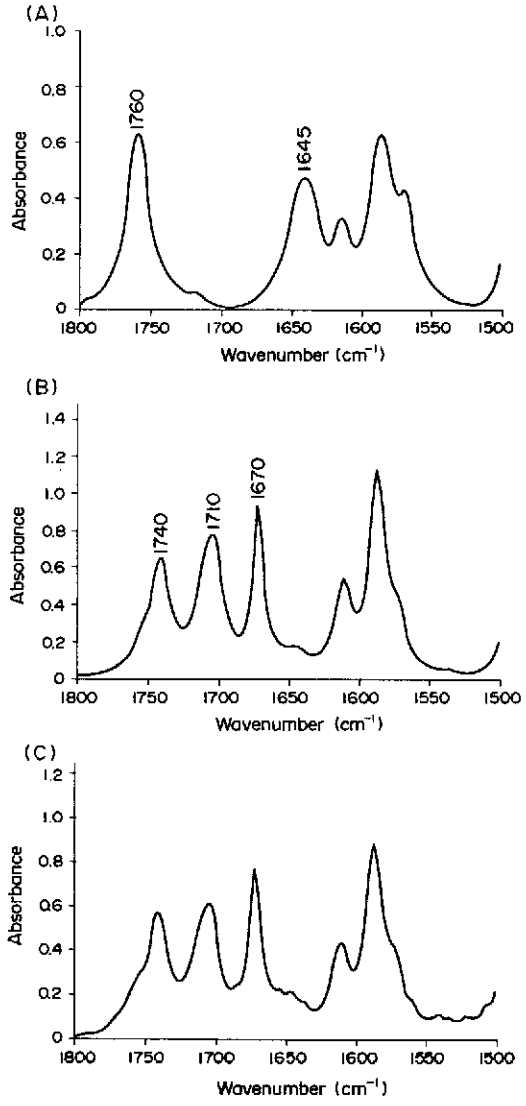

Figure 6
Expanded carbonyl region DRIFT spectra for SC-41930 samples: (A) HM sample, (B) LM sample, (C) LM sample in a capsule formulation.

distinguishing features of the transmission IR spectra of the LM and HM polymorphs were observed in the DRIFT spectra. The only significant difference in the carbonyl region of the DRIFT spectra and transmission IR spectra was the absence of the absorbance at 1760 cm^{-1} in the LM sample, which was the absorbance that was dependent on the duration of the pellet formation period for the transmission IR experiments. It should be noted that the spectral differences observed for the LM and HM forms with DRIFT spectrometry and transmission IR spectrometry were consistent for several lots of each form. Also, the spectral features unique to the LM and HM forms were evident for samples in a capsule formulation containing common excipients such as lactose and hydroxypropyl methylcellulose. The carbonyl region spectra shown in Fig. 6(C) was obtained for an LM sample in a capsule formulation. This suggested that DRIFT spectrometry could be used to study the impact of the formulation process on SC-41930 polymorphism.

Further studies were conducted to determine the suitability of using DRIFT spectrometry for estimating the levels of the LM polymorph in HM samples. A theoretical framework for quantitative measurement with DRIFT spectrometry is provided by the Kubelka–Munk expression:

$$R = 2.303\ EC/S, \qquad (1)$$

where R is the absolute reflectance of an 'infinitely thick' opaque layer measured against the non-absorbing powder, E is the molar absorption coefficient, C is the molar concentration of the analyte, and S is the scattering coefficient. Limitations in the Kubelka–Munk expression and quantitative analyses with DRIFT spectrometry have been investigated and detailed by Murthy and Leyden [18]. The scattering coefficient is dependent on the packing density, particle size, and particle shape of the non-absorbing dispersant. The linear calibration range for DRIFT analyses is usually limited since the scattering coefficient is not solely dependent on the non-absorbing dispersant at higher analyte concentrations. Use of DRIFT spectrometry for a quantitative SC-41930 polymorph determination seemed feasible since the method of preparation and chemical purity of SC-41930 lots would be carefully controlled. Also, physical properties

such as bulk density and particle size would be determined and recorded for each lot.

Calibration studies for quantitative LM polymorph determinations with DRIFT spectrometry were completed by determining the peak area for the absorbance at 1670 cm^{-1}, for HM samples spiked with 0–25% (w/w) of the LM polymorph. The absorbance at 1670 cm^{-1} had been shown to be unique to the LM polymorph. A calibration plot and regression data are shown in Fig. 7. Linear calibration was observed. Based on the calibration data, the detection limit for the LM form in HM samples was estimated to be about 1%. Figure 8 overlays spectra for an HM sample spiked with 5 and 10% of the LM polymorph. Additional quantitative studies were conducted with several spiked and authentic SC-41930 samples, using 5 and 10% spiked samples (LM in HM) as external standards. Three peak area determinations were completed for each standard and each sample. Samples were placed in the DRIFT spectrometry cell for each determination. Peak area data as well as actual and measured levels of the LM polymorphs are summarized in Table 2. The DRIFT method yielded good agreement between measured and actual levels. The RSD values in Table 2 characterize the type of precision that is

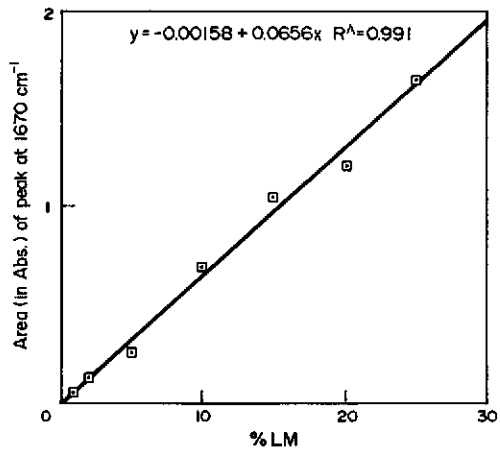

Figure 7
Calibration curve for HM samples spiked with 0–25% (w/w) of the LM samples.

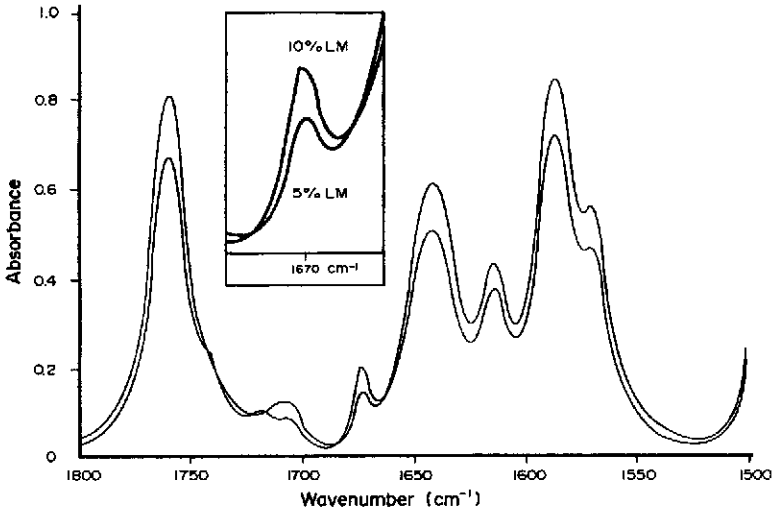

Figure 8
Expanded carbonyl region DRIFT spectra for an HM SC-41930 sample spiked with 5 and 10% (w/w) of a LM sample.

Table 2
Quantitative data for spiked and authentic samples

Day	Peak area 1670 cm^{-1}	RSD (%)	Sample	Measured % LM
1	0.087	14.5	authentic HM	1.4
1	0.689	3.1	spiked HM — 10.5%	10.5
2	0.144	4.7	spiked HM — 2.6%	3.0
2	0.000	0.0	authentic HM	0.0
3	0.335	6.3	spiked HM — 5.1%	5.0
3	5.274	4.5	authentic LM	80.1

achievable for the peak area measurements, using three determinations. Two experimental factors helped improve the run-to-run and day-to-day consistency in results. All DRIFT spectra were generated using spectroscopic grade KCl-dispersant that had been ground for 2 min in a mechanical mixer to provide uniform, consistent particle size. Also, samples were loaded into the DRIFT cell in as consistent manner as possible.

Conclusions

DRIFT spectrometry is particulairly well-suited for IR investigations of drug substance polymorphism and subsequent test development since the requisite preparation procedures do not subject the samples to excessive grinding or pressure. The DRIFT spectrometry method described in the present report provides a valid, convenient approach for identifying the two polymorphic forms of SC-41930, when used in tandem with thermal analysis results. The approach can also be used to provide an estimate of the LM levels in HM samples. It should be noted that the approaches put forth in the present study would not detect an additional polymorph(s) with identical IR spectral and thermal analysis features.

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References

[1] J. Haleblian and W. McCrone, *J. Pharm. Sci.* **58**, 911–929 (1969).
[2] J. Haleblian, *J. Pharm. Sci.* **64**, 1269–1288 (1975).
[3] J. Mullins and T. Macek, *J. Amer. Pharm. Ass., Sci. Ed.* **49**, 245–248 (1960).
[4] S. Khalil, M. Moustafa, A. Ebian and M. Motawi, *J. Pharm. Sci.* **61**, 1615–1617 (1972).
[5] A. Aguiar, J. Krc, A. Kinkel and J. Samyn, *J. Pharm. Sci.* **56**, 847–853 (1967).
[6] Y. Kawashima, T. Niwa, H. Takeuchi, T. Hino, Y. Itoh and S. Furuyama, *J. Pharm. Sci.* **80**, 472–478 (1991).
[7] J. Pearson and G. Varney, *J. Pharm. Pharmacol.* **21**, Suppl., 60S–69S (1969).
[8] M. Moustafa, A. Ebian, S. Khalil and M. Motawi, *J. Pharm. Pharmacol.* **23**, 868–874 (1971).
[9] M. Summers, R. Enever and J. Carless, *J. Pharm. Pharmacol.* **28**, 89–99 (1976).
[10] N. Kaneniwa and M. Otsuka, *Chem. Pharm. Bull.* **33**, 1660–1668 (1985).
[11] M. Otsuka, T. Matsumoto and N. Kaneniwa, *J. Pharm. Pharmacol.* **41**, 665–669 (1989).
[12] H. Ibrahim, F. Pisano and A. Bruno, *J. Pharm. Sci.* **66**, 669–673 (1977).
[13] Y. Takhashi, K. Nakshima, T. Ishihara, H. Nakagawa and I. Sigmimoto, *Drug Dev. Ind. Pharm.* **11**, 1543–1563 (1985).
[14] M. Fuller and P. Griffiths, *Anal. Chem.* **50**, 1906–1910 (1978).
[15] S. Yeboah, W. Yang and P. Griffiths, *Proc. Soc. Photo-Opt. Instrum. Eng.* **289**, 118–120 (1981).
[16] E. Suzuki and W. Gresham, *J. Forens. Sci.* **31**, 1292–1313 (1986).
[17] R. Gimet and A. Luong, *J. Pharm. Biomed. Anal.* **5**, 205–211 (1987).
[18] R. Murthy and D. Leyden, *Anal. Chem.* **58**, 1228–1233 (1986).

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