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Rapid verification of identity and content of drug formulations using mid-infrared spectroscopy

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Abstract: A general method for the rapid verification of both identity and content of complete solid drug formulations has been devised. Infrared spectra for the samples were recorded using the diffuse reflectance technique, and specially written software was employed to identify the type of formulation and level of active ingredient. This software was devised to ensure reliable use when applied by those with minimal operator skills. Three differing drug tablet formulations containing simvastatin, enalapril maleate and lovastatin, as well as a capsule formulation containing finastride were studied. Adequate precision was obtained to reliably verify drug dosage levels. Near-infrared (NIR) and mid-infrared (MIR) spectrometers were evaluated for use with the method. The MIR instrument allowed sufficient resolution and spectral/structural selectivity to reliably verify correctness of either of two near derivative drugs necessarily present in the same clinical study. Drug tablet and capsule dosage levels tested ranged from 0.2 to 40 mg of drug. Approximately 1% (w/w) of the drug in the formulation was the minimum amount determined. Parameters affecting method ruggedness in routine use were optimized. Experimental addition of an extraneous material to a simvastatin formulation was easily detected and flagged by the routine test procedure. Subsequent data retrieval and searching against spectral libraries was used to demonstrate identification of the additive.

Keywords: *Diffuse reflectance spectroscopic measurements; drug verification; Fourier-transform infrared spectroscopy; mid-infrared quantitative analysis; therapeutic efficacy.*

Introduction

In order to demonstrate clinically the therapeutic efficacy, or advantage, of an experimental drug candidate it is often necessary to conduct studies with the concurrent administration of drugs of similar clinical indication (control drugs) or formulations without the active component (placebo drugs). The design of these studies is such that the identity of the dosage forms cannot be visually distinguished (blinded) by the patient or the administering physician. The dosage forms for these studies are manufactured and individually assayed, typically by a selective reversed phase liquid chromatographic (LC) procedure. They are then released to a clinical packaging staff for assembly into specific containers for the multicomponent clinical study. Because of the blinded nature of these materials, an additional expenditure of analytical resource is often required to verify the correctness of the clinical assembly.

The purpose of this study was to evaluate the feasibilities of non-invasive near- (NIR) and mid-infrared (MIR) diffuse reflectance spectroscopic measurements as rapid and routine procedures to verify the correctness of the clinical packaging. Both NIR and MIR spectral region techniques, using advanced software, would generally be expected to perform this function rapidly, and with significant cost reduction, once methods were developed. It was necessary that the method of choice involve minimal operator skills and be as rapid as possible, yet provide a high degree of reliability. It must be understood that this clinical supplies analysis only required quantitation to a degree necessary for the verification of a given dosage level. The dosage forms had been previously assayed, typically by LC, and released unpackaged.

An internal survey, spanning a 3-year interval, indicated that approximately 85% of the clinical materials distributed by the clinical packaging area of Merck, Sharp and Dohme Research Laboratories were solids. Solid state diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) was considered as a viable option for these samples, with the

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exception of high potency formulations in which the active moiety was <0.5% of the total formulation weight. For these high potency formulations, more sensitive ultraviolet spectroscopy, fluorescence, thin layer chromatography (TLC) or LC methods with appropriate solution sample preparation are required. Mid-infrared methods (MIR) usable for verification testing of non-solids have been demonstrated. Hartauer and Guillory [1] used attenuated total reflectance (ATR) to determine propylene carbonate in cream and ointment bases. Urbanyi and Stober [2] analysed creams, inserts, lotions and ointments by FT-IR methods.

Although numerous authors have recently published investigations involving qualitative or quantitative analysis of drugs using IR techniques, no paper has been devoted to an overall identification/verification of both compound and concentration level of interest in application to clinical supply release testing. Typical examples of published reports follow.

In 1989, Hartauer and Guillory [3] discussed analysis of trimethoprim and sulphamethoxazole in a pharmaceutical formulation. Utilizing FT-IR/ATR and partial least-squares analysis of the spectral data, relative quantitative errors of <0.7% were obtained for both drugs. MIR/DRIFTS, using linear stepwise multiple regression analysis, was applied to simultaneous quantitation of ethenzamide, isopropylantipyrine, caffeine and allylisopropylacetylurea by Park *et al.* [4] in 1988. Ficarra *et al.* [5] applied MIR/DRIFTS analysis to formulated benzodiazepines. Good recoveries and reproducibility were obtained according to a study published in 1987.

Near-infrared (NIR) methods have developed rapidly since grain proximate analysis applications of the 1970s [6]. In the last few years in this particular laboratory, NIR has been applied to inventory control of various drug substances with some success and has been suggested as a viable solution to control of packaged clinical supplies. NIR methods, although well-suited to certain types of routine quantitative use in the quality control laboratory, suffer from the apparent inability to distinguish closely related derivatives of certain organic compounds.

Although inappropriate for the final testing operation, an obsolescent MIR instrument was utilized in a feasibility study with the following major objectives: (a) a comparison of the NIR and MIR applications to tablets and capsules using the methods described below, (b) evaluation of MIR versus NIR for minimum measurable amount of the drug in the formulation, and (c) ability of NIR and MIR to distinguish between structural analogues. When the feasibility study was completed and evaluated, a method development study using current commercial FT-MIR and FT-NIR instruments was undertaken with the following objectives: (d) a repeat of objective (c) above, and (e) development of a practical NIR or MIR technique [depending upon the success of part (d)] such that a convenient, durable and reliable IR method would result. The analysis was to be eventually performed by technicians without extensive IR experience. Full data treatment with disk storage was to be allowed in the event that questions arose on particular samples in the clinic. If necessary, spectral stripping using absorbance subtraction techniques could later be employed to identify and quantitate each component. Full utilization of the best software available would be effected.

MIR investigations of drug formulations at Merck have shown that drug moiety bands such as carbonyl stretching and aromatic skeletal in-plane vibration bands are quite consistently observable in the presence of the typical excipients such as lactose, starch, or cellulosic materials. This selectivity for drug bands is apparent on examination of classical MIR drug collections (refer to Sammul *et al.* [7] of the FDA).

Experimental

Materials

The systems investigated in this study were: lovastatin (tablet) — placebo, 20 and 40 mg; simvastatin (tablet) — placebo, 20 and 40 mg; enalapril maleate (tablet) — placebo, 1.25, 2.5, 5 and 20 mg; finastride (capsule) — placebo, 0.2, 0.5, 1, 5 and 10 mg. Refer to Fig. 1 for the structures of these drugs, which were chosen to demonstrate structural selectivity and typical examples of tablets and capsule formulations.

Feasibility study

For the NIR feasibility study, a Pacific Scientific NIRA, Model 6250, spectrometer was used. The instrument was calibrated using Pacific Scientific software (matrix methods) and a series of previously assayed tablet formu-

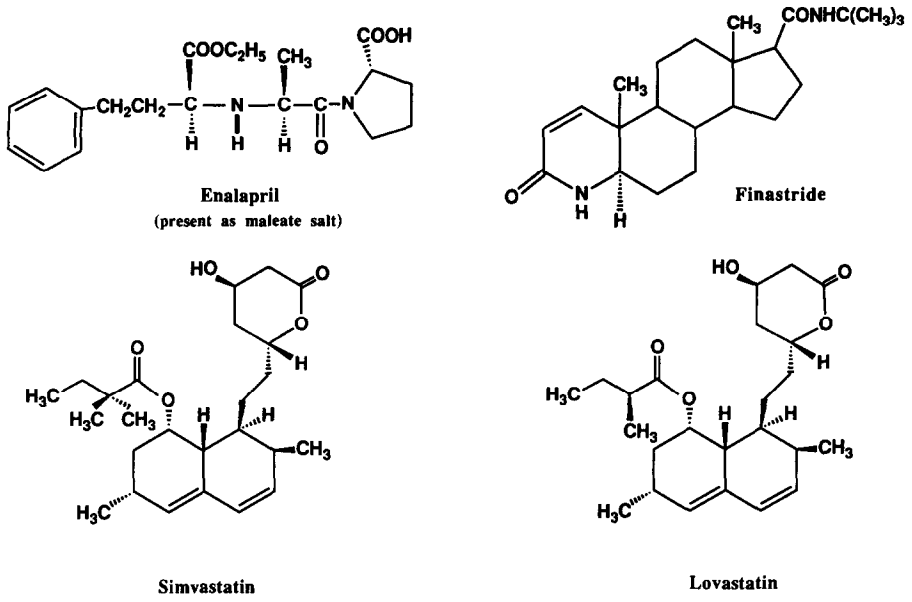

Figure 1
Chemical structures for the drugs lovastatin, simvastatin, enalapril and finastride.

lations (standard powders). The smaller of two available sample holders was employed.

A Bio-Rad FTS 15C/D (Digilab Division, Cambridge, MA, USA) spectrometer was used to record the MIR spectra for the feasibility study. The spectrometer employed a DTGS detector, KBr beamsplitter, and a high intensity Globar source. The conventional KBr pellet technique was applied for analysis, with pellets containing from 1.8 mg of formulation per 200 mg of KBr, to 5 mg of formulation per 500 mg of KBr (for the lower drug concentrations). IR quality KBr (Harshaw Chemical Company, Solon, OH, USA) was used as the pellet matrix and diluent.

Application study

For both the NIR and MIR applications studies, sample preparation involved the milling of the capsule contents and tablets through the use of a Wig-L-Bug grinding mill (Cresent Dental Mfg. Company, Lyons, IL, USA) using disposable containers and balls. The tests utilized a Bio-Rad diffuse reflectance accessory with a quad sample slide for the acquisition of spectra from the powdered samples. The formulation samples were examined without the addition of diluent. Potassium bromide powder (Harshaw–Filtrol, Cleveland, OH, USA) was used as the reference material for background spectra.

A Bio-Rad FTS 7 (Digilab Division, Cambridge, MA, USA) spectrometer was used for the acquisition of MIR spectral data from 4000 to 400 cm^{-1} at 2, 4 and 8 cm^{-1} resolutions in the application study. Sixty-four scans were co-added for each spectrum, taking 1 min at 8 cm^{-1} resolution. It was not known initially what minimal resolution would be needed to adequately define the sharp bands in this region. All resolutions worked adequately for the analyses performed, and, therefore, the 8 cm^{-1} data was used as it required the least amount of time for acquisition. The spectrometer employed a DTGS detector, ceramic source, and a Ge/KBr beamsplitter.

A Bio-Rad FTS 40N (Digilab Division, Cambridge, MA, USA) spectrometer was used to acquire the NIR data from 14,000 to 2900 cm^{-1} at 8 cm^{-1} resolution, 64 scans co-added (1 min collection). Since the bands were not sharp in the NIR region, this was deemed sufficient resolution to define the band positions and shapes. The spectrometer was equipped with a lead selenide detector, tungsten halogen source, and a quartz beamsplitter.

Results and Discussion

Final methodology involved two steps: (1) sample preparation (4–7 min) and (2) analysis (5 min). An accurately weighed portion of the

finely ground formulation powder (following 2–5 min grinding time in the disposable container of the grinding mill) was transferred to the sample cup of the DRIFTS accessory. The cup was placed into one of the four positions in the accessory slide. The analysis procedure was automated to a single push button operation from the keyboard. Initially, the operator was prompted to check that the accessory and sample were in proper placement in the FT-IR spectrometer prior to data collection. The program then requested the technician for entry of the batch number, operator's name, and tablet or capsule weight prior to recording of the IR spectrum. The analysis proceeded with the library search of the collected spectrum for identification and correlation of drug concentration level. A more thorough quantitative analysis for actual measurement of the active drug moiety was then performed. Generation of fixed format hardcopy reports and spectral plots recording the findings followed.

The spectral information reflected by the MIR and NIR regions was quite different. Fundamental vibrations, highly characteristic of a given molecule and well suited for structural elucidation and identification, are observed in the MIR. The NIR absorbances were much weaker, and are based on the overtones and combinations of hydrogenic stretching modes. This latter dependency introduces the constraint that perturbations of anything involving hydrogen bonding, such as moisture content affected by the partial pressure of water vapour around particles, must be controlled otherwise quantitation can be adversely affected. Sample preparation for the NIR required that particle-size uniformity also be controlled carefully, as scattering is greater at the shorter wavelengths of the near-infrared and the spectrum can completely disappear [8].

Another characteristic of the NIR is that it generally quantifies rather than classifies, so there exists a problem in the detection and identification of erroneously presented materials (no discriminant analysis). Because the mid-infrared spectra consist of many sharp bands corresponding to vibrational transitions, characteristic of different functional groups, this task of detection/identification is simplified. Furthermore, because calibration in the NIR is usually accomplished through correlation, care must be taken to ensure that there does not exist accidental involvement of secondary correlations in the calibration equation, such as monitoring lactose rather than the active ingredient.

Initially it was not known which spectral region or regions would permit the accomplishment of the objectives set forth. Therefore, the most difficult situation perceived, verification of identity and content of simvastatin versus lovastatin, was explored. These compounds differ by the presence and absence of a α-methyl group to an ester carbonyl, respectively.

Verification of identity and content of simvastatin and lovastatin was accomplished by observation of the ester carbonyl band contour and position near 1705 and 1700 cm^{-1}, respectively (see Fig. 2). Both MIR and NIR measurements were comparable to a low concentration limit of about 1% (w/w) in the presence of excipients. The NIR technique was deficient in that its use did not allow differentiation between the α-hydrogen atom on the ester function (lovastatin) and an additional α-methyl group (simvastatin). Refer to Fig. 1 for the chemical structures of these drugs. This was a most challenging system, yet typical of the requirements used in clinical supply situations. As NIR was unable to provide a clear spectral difference, particularly at low concentrations (refer to Fig. 3) work using NIR was discontinued and the investigation focused on development of a MIR method.

Adequate identification and quantitation of the active moiety finastride was accomplished using the conventional MIR KBr pellet technique. The range from 0.04 to 200 mg/capsule could be accurately quantitated by measuring the carbonyl absorbance near 1688 cm^{-1}.

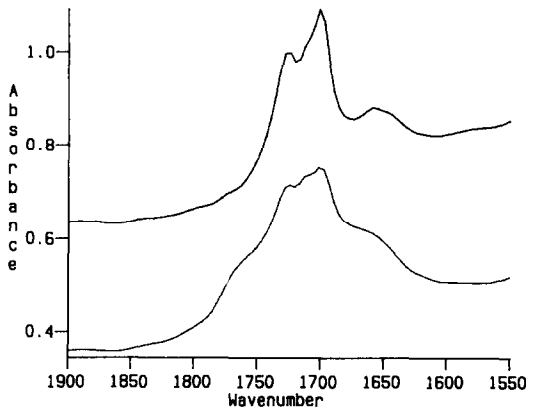

Figure 2
Mid-infrared spectra for the carbonyl region of the drugs simvastatin (bottom) and lovastatin (top) at the 20-mg concentration level.

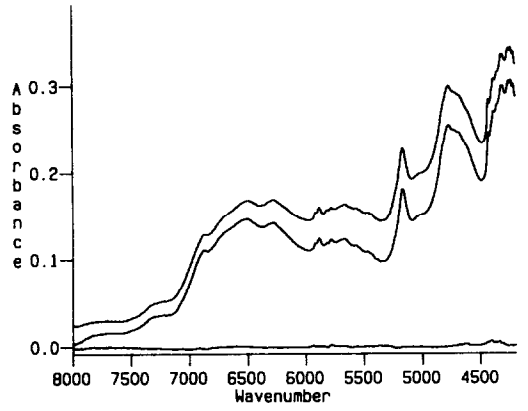

Figure 3
Near-infrared spectra for the 20 mg of simvastatin (top), lovastatin (middle) and difference spectrum (bottom).

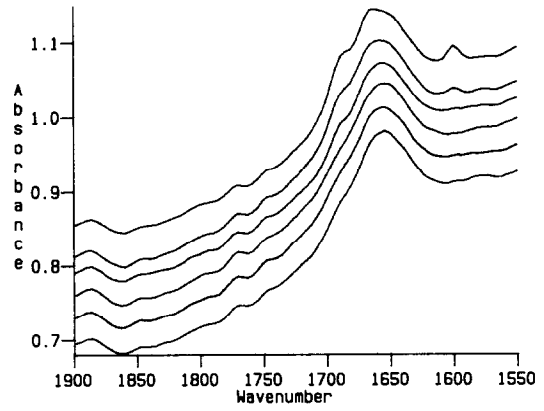

Figure 4
Mid-infrared spectra for the carbonyl region of the finastride drug series placebo, 0.2, 0.5, 1, 5 and 10 mg (bottom to top).

Figure 4 clearly illustrates the dependence of this band intensity upon concentration of the active moiety in the formulation. This measurement was facile — but the preparation of pellets was time consuming and required skill and precision. Because the final method objectives were not met, the KBr pellet method was not seriously considered due to time and skill requirements. However, it did fulfil needs to check MIR structural selectivity and the measurement concentration range for the drug.

In a similar manner, using the pressed pellet technique of the feasibility study, enalapril maleate tablets were measured at low concentrations using the carbonyl stretching band near 1734 cm^{-1} (see Fig. 5).

After establishing that all the samples possessed at least one characteristic absorption

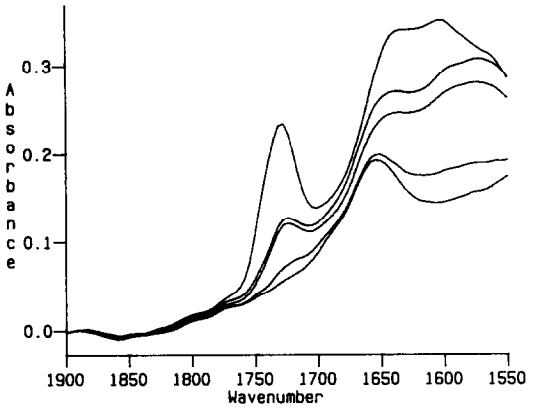

Figure 5
Mid-infrared spectra for the carbonyl region of the enalapril maleate drug series placebo, 1.25, 2.5, 5 and 20 mg (bottom to top).

band in the MIR feasibility study, the collection of spectra for each of the drug formulations was undertaken by DRIFTS. All the samples were examined in duplicate to assure reproducibility, and minimize random errors. This also gave the opportunity to create a library from one set of data, and search the repeat data to verify the ability to distinguish the samples from one another.

A necessary adjunct to the success of technician-performed clinical supply testing was to be its rapid, mistake-proof operation. These considerations were addressed in the following manner. Duplicate tests in the MIR were performed to check the effects of grinding upon identification and quantitation processes. It was found that a grinding time between 2 and 5 min in the Wig-L-Bug apparatus gave reproducible results in both quantitation and identification of the drug formulation examined by DRIFTS. However, if the grinding time was lengthened, the drug identification still remained correct, but the quantitation of the active ingredient became erroneous.

It was found by duplicate runs that the proper identification and content verification of the 5 mg enalapril maleate formulation was not affected by running differing portions of the same powder, overfilling or underfilling the sample cup, nor time delays of up to 12 h duration.

Because specific absorbance bands could be detected and assigned to the active ingredient in each formulation, quantitative methods, with the direct application of Beer's Law, were developed using direct absorbance value

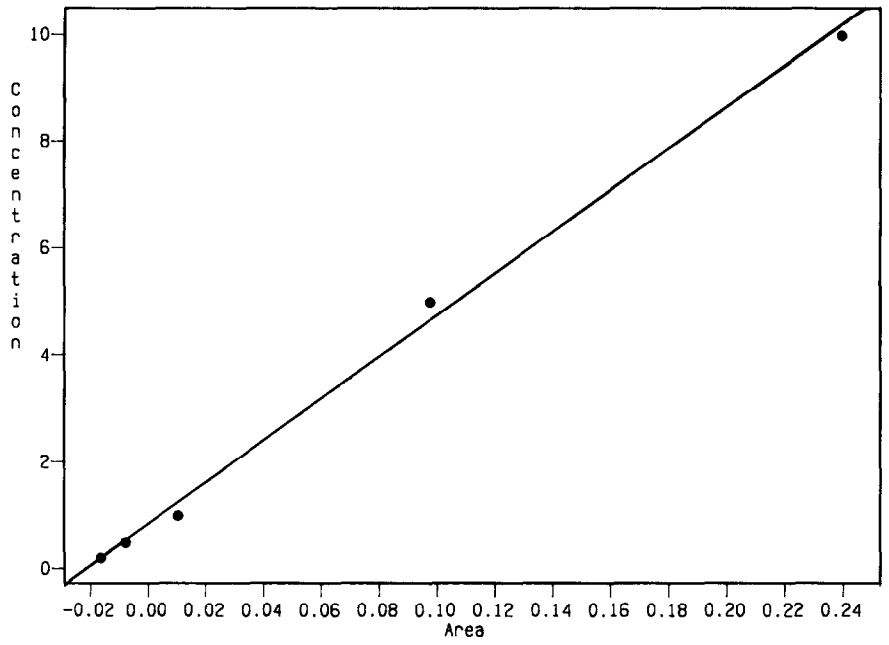

Figure 6
Beer's Law plot of concentration versus area absorbance measurements for the finastride drug series shown in Fig. 4. The measurements shown were the average from duplicate examinations of each of the formulations.

measurements (lovastatin, finastride), band ratio methods (simvastatin), or integrated area methods (enalapril maleate). All the systems investigated produced linear relationships due to the active species. Enalapril maleate was easily detected by MIR/DRIFTS analysis even at the 0.25% level, while finastride was used to demonstrate Beer's Law adherence over a wide concentration range (refer to Fig. 6).

An additional advantage of using the MIR range for monitoring drugs is the acquisition of full spectra which can then be searched for identification. A library limited to spectra of drug formulations present in the clinical packaging area was used. If poor search results occurred, such as a high hit quality index (HQI) value which indicates poor matching, then spectral subtraction would be performed. The procedure removed spectral contributions arising from known ingredients and the resultant residual spectrum could be searched against vast MIR libraries of reference compounds for identification. This can be very important in a case where a spectrum of an interloping species in a formulation can be obtained and then identified by this expanded search technique. Additionally, spectral subtraction can be used for the verification of a product. For example, the subtraction of a product spectrum minus a reference spectrum corresponding to that formulation should yield a flat baseline, with no residuals observable.

It is extremely important to detect and identify any extraneous substances in a formulation before the preparation is released. An experiment was devised to demonstrate the ability of the FT-IR instrument to flag the presence of extraneous materials. In order to accomplish this task, one simvastatin sample was experimentally spiked with 2% (w/w) of a specially selected material. The additive was selected so that it had a very similar spectrum to that of simvastatin. Upon analysis, a high HQI of 1.43 was obtained for the simvastatin formulation, flagging a possible contamination/interloper situation by signalling the need for further analysis. After subtracting the spectral contributions due to the simvastatin formulations, the search readout correctly identified the residual spectrum as due to this added material (refer to Fig. 7), even though the strongest bands in this difference spectrum were less than 0.01 AU. The significance of this analysis cannot be over-emphasized. Even though the noise level in the difference spectrum appeared to mask the spectral features due to the added species, the computer software was able to extract the relevant infor-

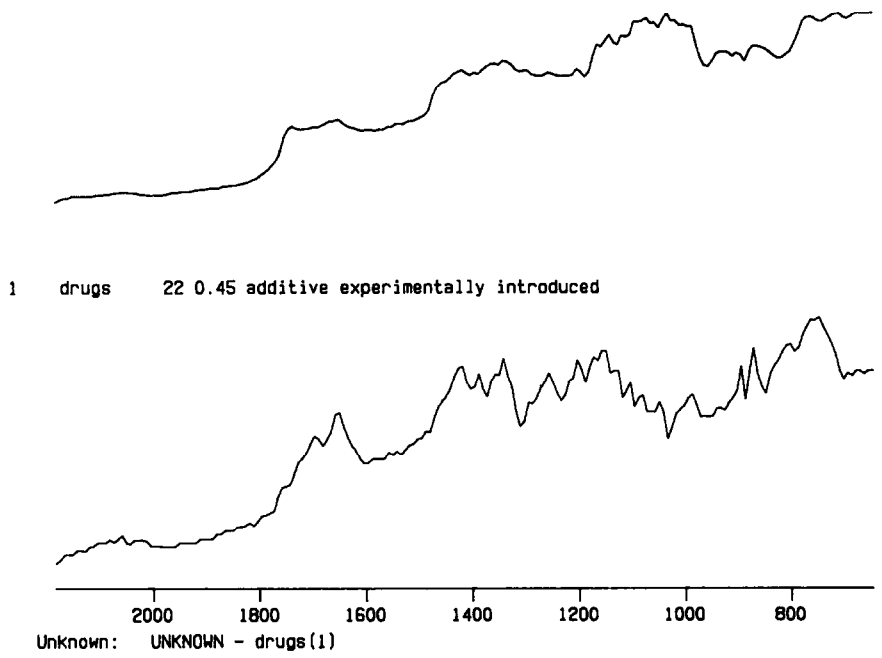

Figure 7
Library search results for an experimentally spiked sample of simvastatin with 2% by wt of a specially selected additive after the simvastatin formulation effects have been removed by spectral subtraction.

mation from the random noise contribution in order to provide positive identification of the material.

Conclusions

Near-infrared spectroscopy could not allow for differentiation between an α-hydrogen atom on the lovastatin ester functionality and an additional α-methyl group in simvastatin, whereas MIR spectroscopy could distinguish between the two similar structures. Studies in the NIR were therefore suspended and the MIR region was used to develop a reliable, rapid, durable procedure for dosage form verification. Sample preparation variables involving DRIFTS cup filling, variance in grinding times, delay between preparation and analysis, duplicate examinations, and the addition of interloping species were explored and, as necessary, boundaries were established for sample manipulation. All the systems investigated produced linear relationships due to the active species using direct absorbance measurements, band ratios, or integrated area methods for MIR analysis. The added advantage of having the capability to detect and identify interloping species, in addition to just requiring a few samples for calibration, made the MIR region considerably more desirable than the correlation quantitation used in the NIR. All the objectives set forth concerning evaluation of MIR versus NIR, definition of minimum measurable amounts of active ingredient, and development of a practical IR technique which was convenient, durable, routine, simple, and reliable for the identification and quantitation of a drug dosage were met.

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