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Original article

Quantitative analysis of bucillamine and its pharmaceutical formulation using FT-IR spectroscopy

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Abstract

A Fourier transform infrared (FT-IR) spectrometric method was developed for the rapid, direct measurement of bucillamine. Conventional KBr-spectra and DRIFTS spectra were compared for best determination of active substance in its tablet formulation. Two chemometric approaches, partial least squares (PLS) and principal component regression (PCR+) methods were used in data processing. Similar results were obtained with both chemometric methods.
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1. Introduction

Infrared (IR) spectrometry provides a useful way for the identification of drugs [1–4]. However, the traditional techniques employed to obtain the IR spectra, such as alkali halides disks, mulls and thin films, are not all the time adequate for quantitative analysis, however the help of Fourier transform (FT-IR) permits continuous monitoring of the spectral baseline and simultaneous analysis of different components of the same sample [5,6].

Quantitative analysis of the component in pharmaceutical preparation by FT-IR spectrometry is based usually upon the Lambert–Beer law. The principal problems are the ingredients presented in the pharmaceutical preparations. A common problem associated with all IR methods is that there are no specific or unique wavelengths of absorption for any of the components. Therefore, the Fourier transform option is superior to the filter option of spectrophotometers because the determination is not based on a single wavelength that is not unique for the analyte of interest.

Bucillamine (BA), *N*-(2-mercapto-2-methylpropionyl)-L-cysteine (Fig. 1), is a novel disease-modifying anti-rheumatic drug [7,8]. Bucillamine is a cysteine-derived analog that contains two sulfhydryl groups. These sulfhydryl groups react with and neutralize toxic oxygen products that participate in the chemical reaction that leads to reperfusion injury, potentially protecting tissue from damage [9]. Taking into account the physicochemical properties are such that development of a sensitive, specific assay requires extreme care and precautions.

An overview for quantitative determination of D-penicillamines is given by Kucharczyk and Shahininan [10]. Most of the methods are based on chromatography: GC electron capture detection and derivatization [11], GC–MS with methyl acrylate derivatization process [12] or HPLC methods in order to increase specificity and sensitivity of the assay [13,14].

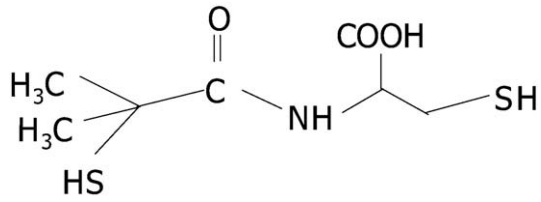

Fig. 1. Structure of bucillamine (BA).

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The aim of the present study is to use FT-IR spectrometry, to investigate the possibility to quantify bucillamine in pharmaceutical preparation, such as Rimatil® 100-mg tablets. The main objective of this work was to develop a chemometric procedure for the fast and accurate determination of bucillamine in pharmaceutical formulations, using PCR+ and/or PLS approaches, reducing the sample pre-treatment and providing direct FT-IR measurement. Conventional KBr-disks or diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) spectra were applied for the analysis of bucillamine.

2. Experimental

2.1. Apparatus

Data acquisition was performed using a Digilab Excalibur FTS 3500 GX FT-IR spectrometer equipped with Merlin version 3.1 (Bio-Rad Laboratories, Sadtler Division, USA). The commercial software used to generate analysis for the principal component analysis was PLSplus/IQ from Galactic Industries Corporation (USA). Microdiffuse reflectance cup and a DRIFTS accessory made by Bio-Rad Laboratories, Sadtler Division, USA were used.

2.2. Reagents and materials

Bucillamine and Rimatil® tablet formulation (containing bucillamine 100 mg per tablet) used for this study was obtained as a gift from Santen Pharmaceutical Co. (Japan). Other excipients used in the tablet formulation are carnauba-wax, hydrogenated oil, sucrose, precipitated calcium carbonate, substituted hydroxy-propylcellulose, cornstarch, povidone, D-mannitol, methylcellulose.

2.3. Analytical procedures

DRIFTS and FT-IR spectra were recorded with different resolutions. The spectra were scanned between 4000 and 400 cm^{-1}, by averaging 64 scans for each spectrum with a resolution of 4 cm^{-1} (data point resolution per interval 1 cm^{-1}) and with a resolution of 8 cm^{-1} (data point resolution per interval 2 cm^{-1}), respectively. In this way we obtained two sets of spectra for each sample. The background spectra were obtained for each experimental condition. Experimental parameters, such as resolutions and calibration methods, (PCR+, PLS1 or PLS2, respectively) were compared and recommendations on the best options for BA analysis were made.

Conventional fused KBr-disk spectra were recorded with a DTGS detector from samples prepared by compressing a 0.3% mixture of standard substance with spectral grade KBr. Using conventional fused KBr-disk, for calibration, the spectra were recorded with a DTGS detector from samples prepared by compressing a standard substance bucillamine (0.5, 1.0, 1.5 and 2.0 mg, respectively) in spectral grade KBr.

For the drug determination the spectra were performed in two different ways:

- Conventional fused KBr-disk spectra were recorded with a DTGS detector from samples prepared by compressing 2.5 mg of drug sample with spectral grade KBr, while the background was spectral grade KBr (BA-KBr).
- DRIFTS samples were prepared by mixing 3 mg of the standard drug with 27 mg of spectral grade potassium bromide. After filling of the cup with a sample-KBr blend, excess material was removed by placement of a microscope slide (with frosted face towards the powder) to minimize the specular reflectance component in the diffuse reflectance spectrum. (BA-DRIFTS).

3. Results and discussion

Figs. 2 and 3 present comparatively the spectra of bucillamine-standard and RAMITIL tablet, respectively, using the two methods mentioned above (BA-DRIFTS) and (BA-KBr).

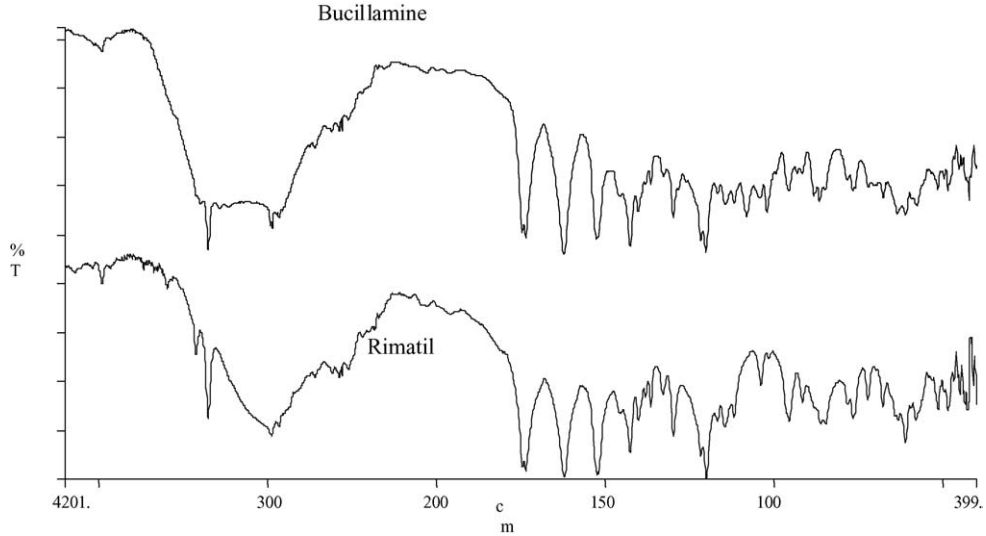

Fig. 2. FT-IR transmittance spectra in DRIFTS procedure.

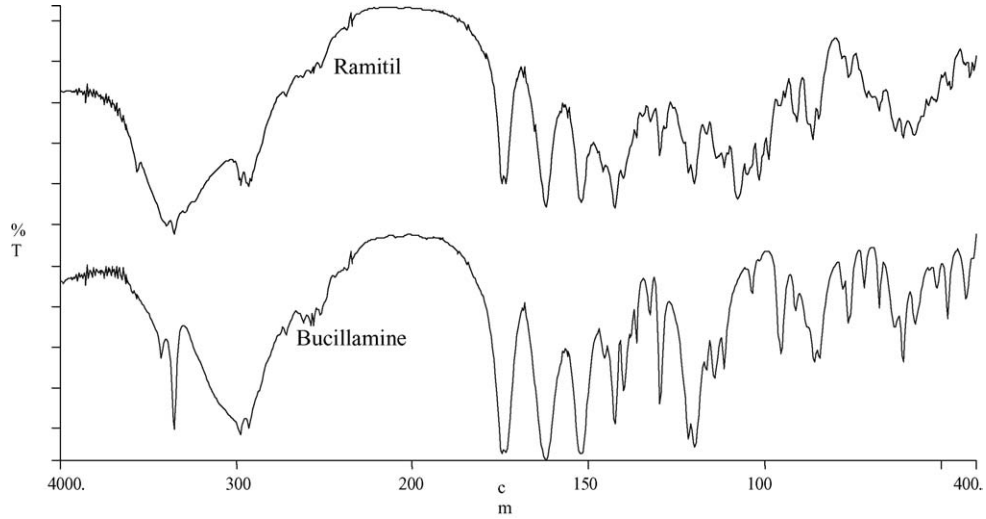

Fig. 3. FT-IR transmittance spectra in KBr-disk method.

As can be seen there is a good similarity between the spectra, in the fingerprint region, (1500–750 cm^{-1}) obtained using the two methods proposed (DRIFTS and KBr-disk).

Determination of the major component in drugs with FT-IR spectrometry provides an enormous amount of spectroscopic information about a sample. Chemometric methods, such as principal component regression (PCR+, improved PCR) and partial least squares (PLS2, multicomponent PLS) analysis are commonly used to extract the specific information relevant to the analyte of interest from the full spectrum [1,15]. These two techniques yields more accurate calibration models compared with multiple linear regression (MLR) where a restricted set of absorption bands is used in the calibration [16].

The projection to latent structures (PLS) regression method was developed by Wold [17] in 1966. A good introduction to the method is given by Geladi and Kowalski [18].

In PCR and PLS2, the spectra are modeled by one set of factors and each property is modeled by relating the concentration values to those factors. In PLS1, the spectra are modeled by a different set of factors for each property and the concentration values are modeled by the respective factors. Hence PLS1 really contains *n* separate calibrations, where *n* is the number of properties in the method.

The data interval were expanded and parts of spectra were eliminated to reduce the size of the data matrix required by the calibration modeling. The calibrations of this study were carried out with the use of the 'expert' option. The first range used was between 4000 and 400 cm^{-1} while the second range was 1800–1475 cm^{-1}. In both cases no blanks were first selected, but after calibration was performed, the computer selects itself ranges of blanks due to the thresholds. The number of data points used for analysis are 3348 and 1109, respectively. Finally, the results are very similar, as can be seen in Table 1, we suggest the use of the PCR+ method, because of the smaller value of R.S.D. (< 2.0%).

RMS error represents the root mean square of error—an overall indicator of the quality of reproduction of the observed spectrum based upon the factors up to the compression point only, it should be of the order of the standard deviation of the noise in the spectrum. The peak-to-peak error gives an indicator of localized anomalies (for example spikes) in the observed spectrum. As a general rule the value should be no more than five times the RMS error if the residuals consist only in random noise. Larger values indicates either features in the predicted sample not present in the calibration set, or features that were in the calibration set but were excluded due to the factor compression cutoff point. The total M-distance ratio is derived from the Mahalanobis statistic D^2 for the unknown sample spectrum. A sample for which the M-distance ratio > 1 might be considered outside the range of the calibration set.

4. Conclusions

The proposed method indicates that FT-IR spectrometry is capable for the direct determination of bucillamine in its

Table 1
Comparison of bucillamine determination in Rimatil® tablets using FT-IR chemometric approaches

	BA-KBr		BA-DRIFTS	
	PCR+	PLS	PCR+	PLS
RMS error	0.03796A	0.03804A	0.03796A	0.03804A
Peak-to-peak error	0.1662A	0.1623A	0.1822A	0.1396A
Total M-distance	0.125	0.125	0.115	0.115
Content (mg per tablet)	95.868	96.204	96.598	91.786
R.S.D. (%) ($N = 5$)	1.85	2.54	1.97	2.73

pharmaceutical tablet formulations, such as Rimatil®. With the commercial software involving chemometric approaches, PCR+ and/or PLS, the method proposed by us is simple, precise and not time consuming compared to the chromatographic methods existing in the literature. Quantification could be performed in about 5–10 min, including the sample preparation and spectral acquisition.

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