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Rapid and sensitive determination of Ambroxol Hydrochloride Injection by Raman spectroscopy combined with chemometric models

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Abstract A procedure for the quantitative determination of ambroxol hydrochloride in its commercial injection based on conjunction of Raman spectroscopy and chemometrics is described. The active component constitutes less than 1% (w/v) of the injection in our study. The efficiency of various spectra treatment procedures, including classical least squares (CLS), partial least squares (PLS), principal component regression (PCR) and stepwise multiple linear regression (SMLR), was compared. First, the calibration models were built using ambroxol hydrochloride standard solutions. To compare the predictive ability of the four models constructed, the performance indices were calculated. As a result, both the CLS model and the PCR model were comparably effective ones, of which the difference values were 94.9% and 94.2%, respectively, and the root mean square errors (RMSEs) were 0.07 and 0.08, respectively. Eleven commercial injections were quantified directly applying the developed models. SPSS software was used to compare the difference between the results obtained from the pharmacopoeial HPLC method and Raman analysis, and there was no significant difference between them (p>0.05). It shows that the proposed procedure based on the chemometric treatment of Raman spectra can be a specific, fast and convenient alternative to the compendial qualitative and quantitative determination of Ambroxol Hydrochloride Injection.

Keywords*:* Raman spectroscopy; Ambroxol hydrochloride Injection; Quantitative determination; chemometric models

Introduction

Ambroxol hydrochloride (Fig. 1) is a mucolytic agent used in the treatment of respiratory diseases associated with viscid or excessive mucus. This substance is a mucoactive drug with several properties including secretolytic and secretomotoric actions that could restore the physiological clearance mechanisms of the respiratory tract, which plays an important role in the body's natural defense mechanisms. Ambroxol hydrochloride could stimulate synthesis and release of surfactant by type II pneumocytes. The surfactants act as an anti-glue factor by reducing the adhesion of mucus to the bronchial wall, in improving its transport and in providing protection against infection and irritating agents[1]. Ambroxol hydrochloride is used in various drug formulations, including tablets, capsules, drops, injection, etc. It is the active ingredient of Mucosolvan, Mucobrox, Lasolvan, Mucoangin, Surbronc and Lysopain. Pharmacopoeial method applied for the qualification and quantification of ambroxol hydrochloride is HPLC method [2].

Raman spectroscopy is an effective analytical method in the quantification of components in complex mixtures, including pharmaceuticals [3]. This technique enables analysis of medicines in the form of tablets, capsules and solutions. It is a fast technique compared to other techniques such as classical chromatography. Most of time a spectrum can be collected and then analyzed within several minutes. Unlike many other classical analytical methods, this technique does not require any special sample preparation, which can simplify and shorten the analytical procedure. Contrary to NIR spectra, Raman spectra have the advantage of revealing specific peaks of active pharmaceutical ingredients (APIs) directly and quantifying APIs in solutions. Furthermore, the detailed pharmaceutical composition is not known in the experiment

sometimes. In such a case, it is feasible for Raman technique to build a simplified calibration model based on standard solutions containing only APIs, while NIR analysis did not behave excellently. In spite of these advantages, application of Raman spectroscopy for quantification of APIs, especially for those contained in less than 10% of constitutes was not widespread [4]. Quantitative Raman studies on injection solutions are even rarer though this method can be employed to determine API quantitatively in their intact forms which can significantly simplify the analysis [5, 6].

In the present work, the results of Raman quantification of commercial injection solutions containing only about 0.75% (w/v) of ambroxol hydrochloride from different pharmaceutical companies in China are presented.

Experimental

Materials and methods

The substances used, namely ambroxol hydrochloride, methanol, sodium hydroxide, hydrochloric acid, citric acid and sodium dihydrogen phosphate were of pharmacopoeial or analytical purity. Aqueous solutions were prepared using purified water with resistivity >18MΩcm. Eleven injections of ambroxol hydrochloride (A1–A11) from three different pharmaceutical companies containing a declared 7.5 mg/mL of API were purchased in local pharmacies. Ten standard solutions were prepared by dissolving ambroxol hydrochloride in water, of which the concentration ranged from 5 to 10 mg/ml. The blank sample solution was prepared by mixing all constituents except API at the suitable weight ratios according to recipe from the companies. For recovery test, six solutions with different proportions of API were prepared by mixing all constituents according to the recipe. One batch of commercial

injection was selected for repeatability test.

Apparatus

All spectra were recorded using a DXR Raman Microscope spectrometer from Thermo Fisher Scientific Inc. with an Olympus BX51 Objectives and XYZ 3D automatic platform. The spectral resolution is 2cm^{-1}. A high brightness semiconductor laser at 780 nm with a power of 24 mW was used as the excitation source. The aperture slit is 25μm. The Raman spectra of all the solutions were obtained directly from 3400 to 50 cm^{-1} accumulating 50 scans per spectrum.

Chemometric models

Nicolet TQ Analyst chemometric software was used to construct classical least squares (CLS), partial least squares (PLS), principal component regression (PCR) and stepwise multiple linear regression (SMLR) models and to perform the quantitative analysis of API in commercial products and recovery test solutions. The software could automatically choose eight calibration standards and two validation standards among the standard solutions. Meanwhile, it provided two important performance indices (PI), % Difference and RMSE.

%Difference is calculated according to the following equation:

$$\%Difference = (1 - \sqrt{\frac{\sum_{i=1}^{n} \frac{|C_i^C - C_i^A|}{Er}}{n}}) \times 100 \quad (1)$$

where C^A is the actual component content, C^C is the concentration calculated from Raman data analysis, n is the number of total validation standards, and Er is the difference between the maximum and minimum concentrations for the component in any standard.

RMSE is calculated according to the following equation:

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(C_i^C - C_i^A)^2} \qquad (2)$$

where C^A is the actual component content, C^C is the concentration calculated from Raman data analysis, and n is the number of total validation standards.

If the Performance Index Algorithm is set to "% Difference", the performance indices will range from 0 to 100. The higher the performance index gains, the closer the calculated concentration values are to the actual values. The root mean square error is reported, if the Performance Index Algorithm is set to "RMSE". The closer the RMSE value is to zero, the smaller is the difference between the calculated concentration values and the actual values.

Results and discussion

In Fig.2 Raman spectra of ambroxol hydrochloride, the standard solution, the commercial injection and the blank sample solution are presented. Ambroxol Hydrochloride Injections can be identified and quantified at the same time. The Raman spectra of all eleven analyzed injections denoted A1~A11 are presented in Fig.3.

As the API concentration of the samples was only about 0.75% (w/v), the Raman characteristic peaks of API were not very strong, and only the peak near 1040 cm^{-1} could be distinguished (Shown in Fig.2), and therefore, the spectral ranges including 1040 cm^{-1} were chosen. Furthermore, Specific spectral range for each chemometric model was modified according to the PI index and the correlation coefficient. The spectral range of 1105cm^{-1}~983cm^{-1} was applied in the PLS chemometric model construction, and the range of 1052cm^{-1}~1031cm^{-1} was applied both in the CLS and the PCR chemometric models constructions. The SMLR chemometric model was obtained from the whole spectral range. Typical calibration curves calculated for the

studied API are shown in Fig. 4. In Table 1 the relative errors(R) and the performance indices ("% Difference" and RMSE) for the calibration models are quoted. These values showed that the CLS and the PCR chemometric model were comparably efficient models.

Based on the four calibration models, the eleven studied injections were quantified. The amount of ambroxol hydrochloride determined by Raman analysis is collected in Table 2. The results of reference analysis [2] are also presented in the last column of Table 2. These values showed that both the CLS and the PCR chemometric model were comparably efficient in the case of actual samples. The results obtained from the SMLR and the PLS model didn't match those obtained from HPLC.

The recovery data and repeatability data are presented in Table 3. It showed that there were distinctive differences among four models. The SMLR model seemed to be the poorest one in both recovery and repeatability results. The PLS model performed the best in repeatability test, but the recovery result was not as good as those of the CLS model and the PCR model.

SPSS software was used to compare the difference between the results obtained from compendia HPLC and Raman analysis. The results of the Paired T Test were listed in Table 4. These values showed that there were significant differences between the HPLC method and the PLS or the SMLR chemometric models. On the contrast, there were no significant differences between the HPLC method and the CLS or the PCR chemometric models (p>0.05). Although CLS is one of the simplest calibration methods, it is seldom evaluated during a typical chemometric method development regimen. It may be an ideal calibration method for noise-free spectra, as long as the measured spectra are additive in the pure components [7]. As a result of basing on the

whole spectral range for the SMLR model and rather low concentration of the API, the absorbance of ambroxol hydrochloride was submerged in those of excipients, which led to low efficiency of the SMLR model.

Conclusions

Both the CLS model and the PCR model were comparably effective methods for the quantitative determination of Ambroxol Hydrochloride Injection. This study confirmed the merit of Raman spectroscopy combined with chemometric methods in the quantitative determination of injections without adding internal standard [5, 6]. It showed high potential that Raman spectrometry could be a convenient, fast, non-destructive and reliable method for analyzing injections.

Acknowledgement

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Table 1
The correlation coefficients and the performance indices for ambroxol hydrochloride determination

Method	Corr. Coeff.	PI(%Difference)	PI(RMSE)
CLS	0.99574	94.9	0.0708
PLS	0.99881	92.2	0.107
PCR	0.99574	94.2	0.0793
SMLR	0.99992	71.4	0.349

Table 2
Results (%) of FT-Raman analysis and HPLC

Injections	Batch	CLS	PLS	PCR	SMLR	HPLC
A1	2010040401	102.8	93.6	102.7	89.0	100.5
A2	2010040302	102.5	100.3	102.5	85.9	101.2
A3	2010040301	96.9	97.2	96.8	89.2	101.7
A4	100650	102.3	84.7	102.2	64.9	100.3
A5	071136	106.7	83.4	106.7	72.7	97.4
A6	1007109	101.4	84.3	101.3	70.5	98.7
A7	0712185	100.3	80.7	100.2	64.5	99.8
A8	0712186	104.1	81.8	104.0	61.7	97.1
A9	127252	100.0	93.9	99.9	83.4	101.2
A10	127261	100.7	90.1	100.6	86.9	101.0
A11	127351	105.3	94.4	105.3	89.0	101.2

Table 3
The recovery data and repeatability data (n=6)

No.	Methods	Recovery（%）	Repeatability（%）
1	CLS	99.2±0.8	102.0±3.3
2	PLS	98.7±2.8	96.1±2.6
3	PCR	98.6±2.8	102.0±3.4
4	SMLR	79.5±2.9	91.4±4.1

Table 4
The parameters of the Pair T Test

Pair	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
HPLC-CLS	-2.08	3.84	1.16	-4.66	0.50	-1.80	10.00	0.10
HPLC -PLS	10.52	5.62	1.69	6.74	14.29	6.21	10.00	0.00
HPLC-PCR	-2.01	3.87	1.17	-4.61	0.59	-1.72	10.00	0.12
HPLC -SLMR	22.04	10.02	3.02	15.31	28.77	7.30	10.00	0.00

Figure captions:

Fig. 1. Chemical structure of ambroxol hydrochloride

Fig. 2 FT-Raman spectra of ambroxol hydrochloride (bottom), 7.5mg/ml standard solution (top), one of analyzed injections (middle top), and blank sample solution (middle bottom).

Fig. 3. FT-Raman spectra of eleven analyzed injections

Fig. 4. Calibration curves for ambroxol hydrochloride content obtained for CLS(A), PLS(B), PCR(C) and SMLR(D).

Fig 1

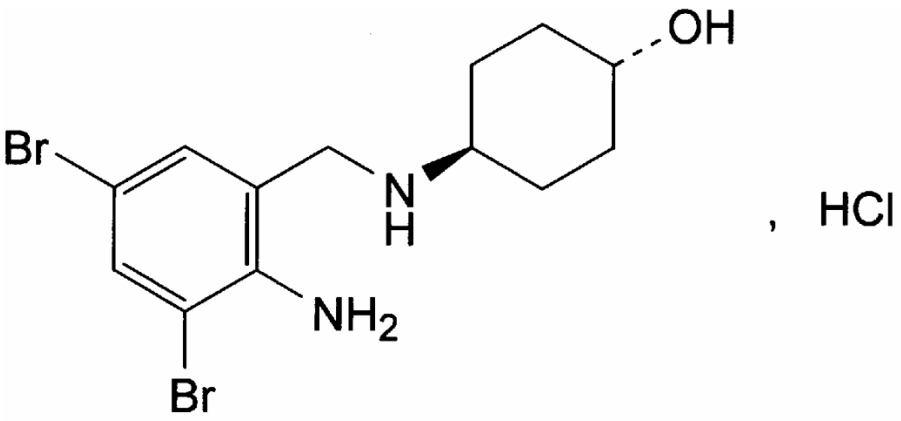

Fig 2

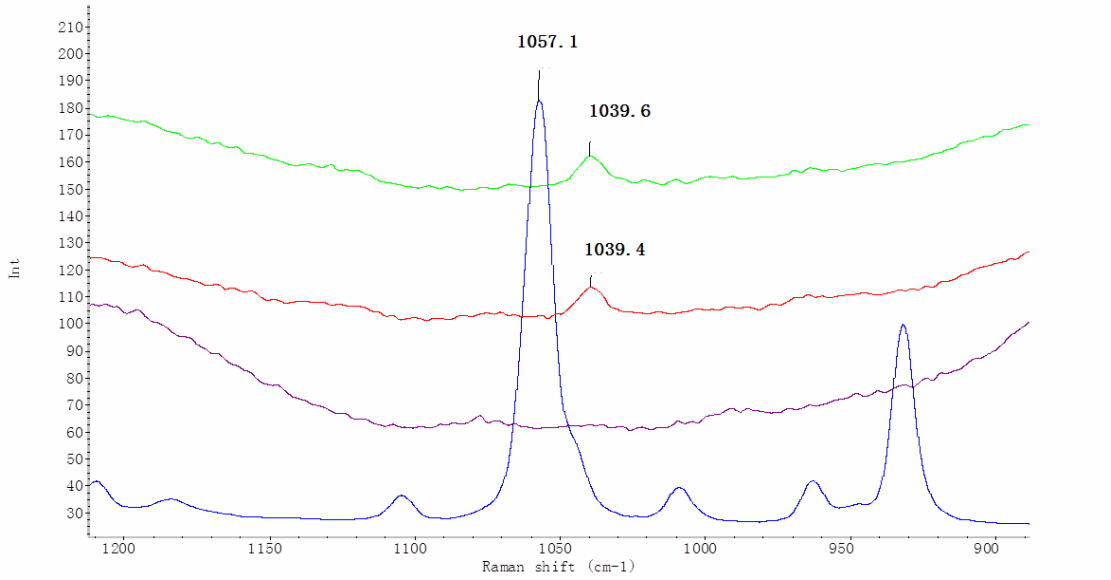

Fig 3

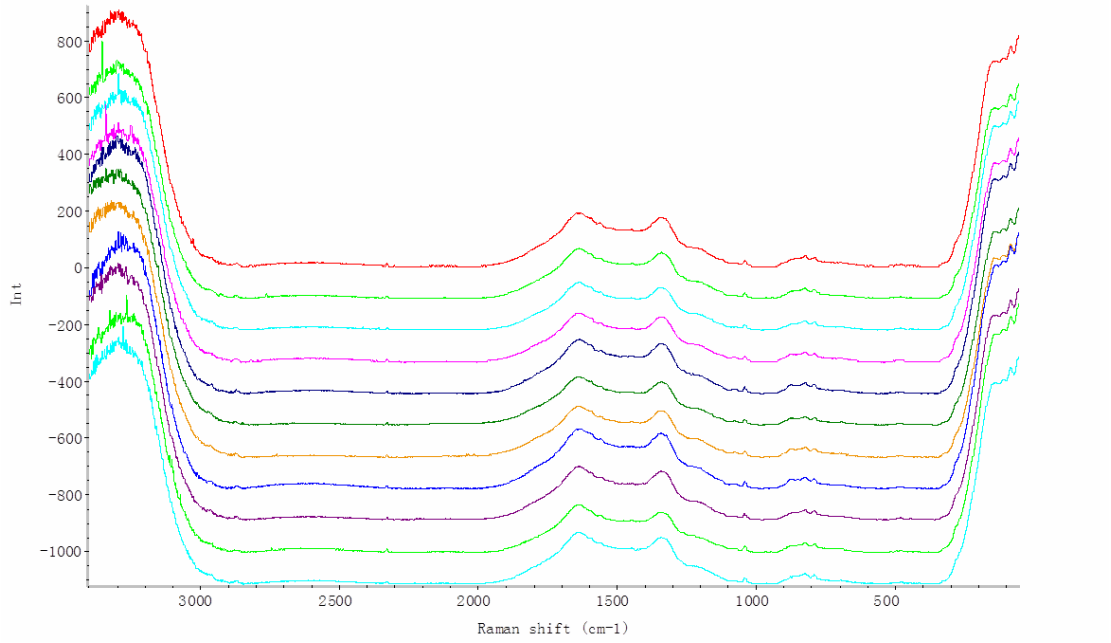

Fig 4

Fig 4A

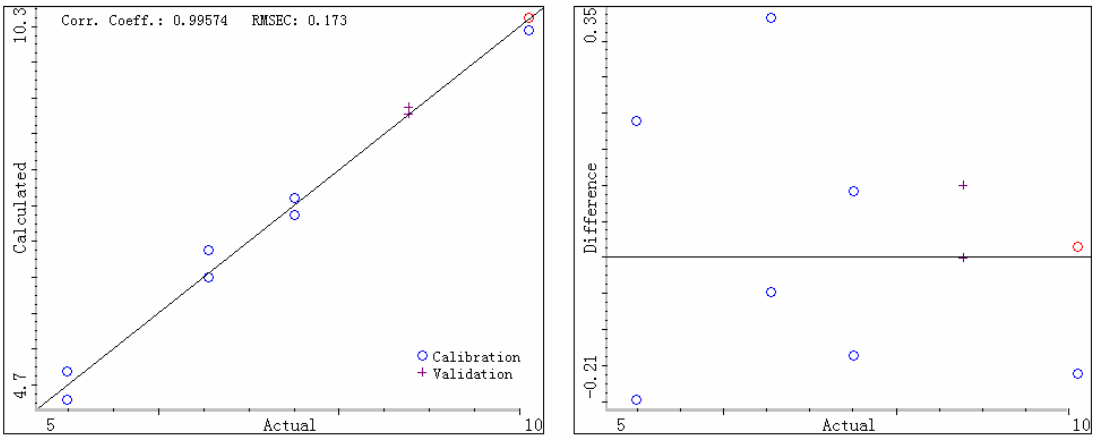

Fig 4B

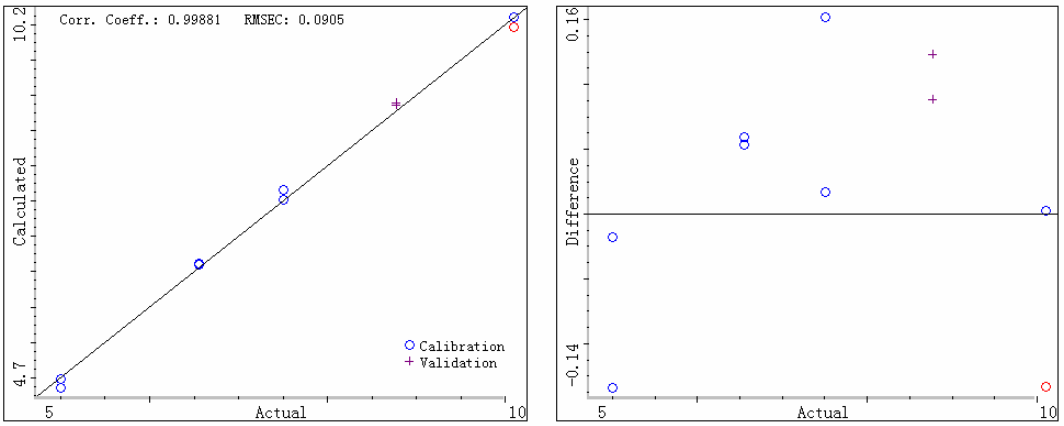

Fig 4C

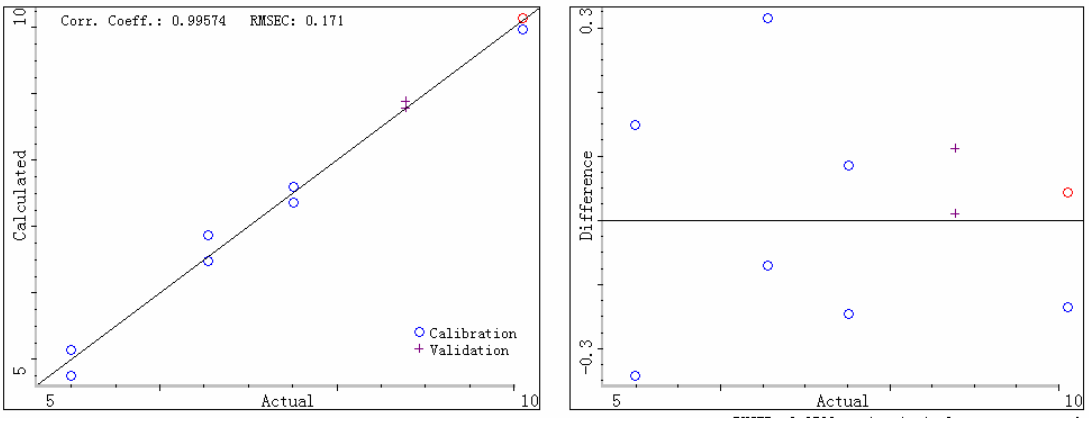

Fig 4D