# Feasibility analysis of lower limit of quantification of NIR for solvent in different hydrogen bonds environment using multivariate calibrations

Zhisheng Wu, Min Du, Chenglin Sui, Zhaozhou Lin, Xinyuan Shi\*, Yanjiang Qiao\*,

School of Chinese Materia Medica

Beijing University of Chinese Medicine

Beijing 100102, P. R. China

vjqiao@263.net; shixinyuan01@163.com

Abstract—Near infrared (NIR) transmission spectroscopy has been widely used for quantitative analysis in different solvents system. Lower limit of quantification (LLOQ) of NIR in solvent of different hydrogen bonding capability has not been reported. This paper was to investigate the LLOQ of NIR for citric acid in different solvents (ethanol, water, acetone, DMSO) combined with partial least squares (PLS) model and accuracy profile. The parameters of PLS model were optimized by cross-validation. PLS model in each solvent was validated using accuracy profile. The result showed that ethanol was the best quantitative solvent for citric acid, and the LLOQ was 2.791 mg/mL. In water, acetone and DMSO, the LLOQ was 4.542 mg/mL, 3.187 mg/mL and 6.563 mg/mL, respectively. This study provided approximate reference values on the LLOQ of NIR in different solvents system.

#### Keywords- Near-infrared spectroscopy; Accuracy profile; Lower limit of quantification; Solvent

#### I. INTRODUCTION

Near infrared (NIR) spectroscopy has been widely used as a powerful technique because its fast, low cost and nondestructive characteristic. Its effectiveness for both qualitative and quantitative analysis has been proven in different fields such as agriculture, food, chemical and petroleum industry [1, 2]. In addition, U.S. FDA recommends the pharmaceutical industry to apply NIR spectroscopy to obtain quality information of drug products [3]. NIR spectroscopy has been accepted within the pharmaceutical industry for raw material testing, product quality control and process monitoring etc. [4].

However, NIR suffer from its high detection limit, trace analysis is still a challenge for NIR analysis. The main difficulty of NIR is overlapping and broad absorption bands due to their overtones and combination tones of hydrogen bonds such as –CH, –OH, –SH and –NH bonds [4-6]. Thus strong self-absorption of solvent may seriously interfere with the absorption signal of solute. NIR transmission spectroscopy has been widely used in different solvents system. It is necessary to investigate the lower limit of quantification (LLOQ) of NIR in solvent of different hydrogen bonds.

In this work, citric acid dissolved in solvents with different hydrogen bonds was analyzed by NIR combined with multivariate calibration and accuracy profile. Water, ethanol, acetone, and dimethyl sulfoxide (DMSO) were selected as typical solvents, which were corresponding to O-H bond, both O-H bond and C-H bond, C-H bond, C-H bond, respectively. Calibration model was set up by partial least squares (PLS) [7, 8]. After the development of PLS model in each solvent, model validation should be performed to scrutinize the accuracy by considering both systematic and random errors. We were interested in estimating the accuracy profile of the analytical model from validation data obtained in different solvents. Based on  $\beta$ -expectation tolerance intervals, the accuracy profile demonstrated the ability of the proposed model to assess the analytical properties in term of accuracy, trueness, precision, lower limit of quantification (LLOQ), range and linearity [9-13]. Finally, this study provided approximate reference values on the LLOQ of NIR in different solvents system.

#### II. MATERIALS AND METHODS

## A. Materials

Citric acid was purchased from Beijing Chemical Works (China). Ethanol, acetone and DMSO were purchased from Concord Technology Co., Ltd. (Tianjin, China). Deionized water was purified by Milli-Q water system (Millipore Corp., Bedford, MA, USA).

## B. NIR equipment and software

The NIR spectra were collected in the transmission mode using an NIR system with Thermonic resulting software (Thermo Nicolet Corporation) over a wavenumber ranged 4000  $\text{cm}^{-1} - 10,000 \text{ cm}^{-1}$ , using 32 scans, 16 cm<sup>-1</sup> resolution per spectrum and recorded as absorbance with air as the reference standard. The sample was hold in a circular sample cuvette with plastic cap (8 mm in diameter). The average spectrum from each triplicate measurement as the final spectrum of each sample was used for quantitative analysis.

# C. Prepatation of sample solutions

Four items of citric acid with the same quantity were weighed accurately into a 500 mL volumetric flask separately, and diluted with each solvent to volume as 4 stock solutions (containing 20 mg of citric acid per mL). Some percentages of 4 stock solutions were transferred to 25 mL volumetric flasks separately, and further diluted with each solvent to obtain different concentrations for establishment of the calibration sets and validation sets.

### D. Model Calibration

The optimum preprocessing method was selected based upon the lowest PRESS to set up PLS model. Cross validation was used with a segment size of five, and PRESS plot was produced. Usually, the first minimum point on the PRESS plot was used to determine the optimum number of factors with the best prediction for the cross validation samples. Data analysis was performed by home-made routines programmed in MATLAB code (MATLAB, The MatWorks, Massachussetts). The calculation of the accuracy profile based on the external validation set results were given by e. noval V3.0 (Arlenda, Liège, Belgium).

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#### III. RESULTS AND DISSCUSSION

#### A. Comparisons of different spectral pretreatment methods

Figure 1 plotted on the PRESS as a function of latent factors for determining citric acid contents in each solvent vs different spectral preprocessing methods. Raw spectra were superior to other spectral preprocessing methods for PLS model.



(\*raw: raw spectra, 1st: First derivative, 2nd: Second derivative, sg: Savitzky-Golay)

Figure 1. Effect of number of PLS latent factors on PRESS values in the range of NIR spectra ( $4000 \text{ cm}^{-1}$  –10,000 cm<sup>-1</sup>).

## B. Prediction results of the NIR method

Figure 2 showed the result of PLS model in each solvent. Each resulting model showed different consistencies between the NIR predictions and reference results in each solvent. The result demonstrated that ethanol is the best quantitative solvent for citric acid, which produced the lowest  $R^2$ , RMSECV and RMSEP (Table I). In pure C-H system (acetone), the result implied that the precision and accuracy showed relatively low potential for quantification of citric acid. The RMSEP and  $R^2$  were corresponding to 0.467 mg/mL and 0.9736. In addition, the model for water and DMSO solvents showed worse agreement between NIR predictions and reference results for both calibration and validation sets, as shown in Table I.



Figure 2. The result of PLS model in each solvent.

TABLE I. CALIBRATION AND PREDICTION SPECIFICATIONS FOR CITRIC ACID CONCENTRATION IN ASSESSMENT OF DIFFERENT HYDROGEN BONDS

Solvent	Factor	R <sup>2</sup> <sub>cal</sub>	R <sup>2</sup> val	RMSEC	RMSEP
Water	14	0.9965	0.9478	0.239	0.656
Ethanol	10	0.9991	0.9962	0.123	0.182
Acetone	5	0.9924	0.9736	0.352	0.467
DMSO	9	0.9702	0.9414	0.694	1.2

### C. Validation the models

Figure 3 displayed the accuracy profile of PLS model in each solvent. The acceptance limits were set at  $\pm$  15% while the maximum risk to obtain results outside these acceptance limits was set at 10%. The Figure 3 (a) indicated that  $\beta$ expectation tolerance limits crossed the acceptance limits in ethanol when the citric acid content was lower than 2.791 mg/mL. As to acetone, the performance of PLS model was relatively large error, as shown in Figure 3(b). The relative error attach to 70%. In addition, the total error of model performance in water and DMSO was larger than that in ethanol and acetone (Figure 3(c) and Figure 3(d)). The result based on the accuracy profile signified that solvent affected the relative error of NIR model performance.

Moreover, analytical properties of PLS model were described in terms of precision, range, linearity etc. Table II showed the ICH Q2(R1) validation criteria of PLS model in each solvent. Bias, repeatability, intermediate precision, risk were larger and more unstable in water and DMSO than in ethanol and in acetone. Ethanol was the best quantitative solvent for citric acid. Its LLOQ was 2.791 mg/mL. In acetone, water and DMSO, the LLOQ was 3.187 mg/mL, 4.542 mg/mL and 6.563 mg/mL. The results suggested that solvent affected other analytical properties.

Each Linear regression model of PLS model in each solvent was fitted on the results as a function of the introduced concentrations in order to obtain the following equations: for ethanol, the regression equations were expressed as Y =0.02276 + 0.9882 X with R<sup>2</sup>=0.9962; for water, Y = 0.2888 + 0.9376 X with R<sup>2</sup>= 0.9471; for acetone, Y = -0.1079 + 1.005 X with R<sup>2</sup>= 0.9848; for DMSO, Y = 0.1842 + 0.9748 X with R<sup>2</sup>= 0.9671. The linearity of the results obtained in each solvent was demonstrated the difference of each model performance. It described the different total errors of PLS model in different

ICH Q2(R1) validation criteria (A)						
		Ethanol			Water	
	LEV	MIC	REB	LEV	MIC	REB
	1.16	1.12	4.39	1.04	1.12	-27.83
	1.99	2.03	-2.84	2.47	2.03	34.29
6	3.17	3.20	-1.41	3.18	3.20	4.40
les	4.27	4.40	-3.86	4.52	4.40	-1.08
net	5 59	5 57	2.21	5 78	5 57	0.16
Tr	6.80	6.73	1.66	6 76	677	2.25
	7.91	7.97	-1 77	7.68	7.97	-7.57
	0.32	9.36	-1.77	0.11	9.36	-7.85
	1.52	/	4 30	/	/	-2.05
	IEV	REP	INP	IEV	PEP	INIP
	1.16	11.40	11.40	1.04	67.11	71.99
	1.10	11.40	5 25	2.47	26.24	26.24
	1.99	4.24	3.23	2.47	14.99	14.99
ion	3.17	3.04	3.04	3.18	14.88	14.88
cisi	4.27	3.15	3.20	4.52	10.91	10.91
Te	5.59	2.23	2.23	5.78	4.89	4.89
	6.80	2.33	2.48	6.76	4.10	4.17
	7.91	2.06	2.06	7.68	6.91	6.91
	9.32	1.79	2.00	9.11	7.20	7.20
	/	/	/	/	/	/
	LEV	RTL	RIS	LEV	RTL	RIS
	1.16	[-16.96, 25.74	41] 44.86	1.04	[-164.8,109.2]	81.07
	1.99	[-13.32, 7.65	] 14.58	2.47	[-33.57,102.2]	73.32
y.	3.17	[-8.23, 5.41]	2.85	3.18	[-23.45,32.25]	23.98
Irac	4.27	[-9.87, 2.14]	4.77	4.52	[-21.51,19.35]	10.77
ccu	5.59	[-1.96, 6.39]	0.34	5.78	[-8.99, 9.32]	0.24
A	6.80	[-3.05, 6.37]	0.53	6.76	[-5.59,10.10]	0.12
	7.91	[-5.61, 2.08]	0.15	7.68	[-20.51,5.38]	5.76
	9.32	[-5.11, 2.24]	0.05	9.11	[-15.62,9.92]	1.88
	/	/	/	/	/	/
	LLO	LLOO ULOO		LLOQ		JLOQ
	2.79	1 9	.930	4.542		7.932
		ICH	O2(R1) vali	dation cri	teria (B)	
		Acetone	22(111) vui	uution on	DMSO	
	LEV	MIC	REB	LEV	/ MIC	REB
	1.00	0.97	19.74	1.80	1.10	63.48
	2.01	1.96	-12.41	2.22	2.21	0.25
	3.47	3.27	-3.56	3.77	3.57	5.77
ess	5.01	4 42	-1 21	4 4 1	4.82	-8 49
nen	5 70	5.42	-2.89	5.78	6.02	-12 30
Tn	677	6.40	-2.39	7 22	7.23	1 33
	0.// 0.//	7 52	-3.75	1.55	9.12	6.40
	0.27	832	-2.71	0.03	804	1.15
	9.12	0.33	0.01	9.04	0.74	1.1.5
	9.74	9.37	0.81	9.70	9.37	1.34

1.00

2.01

3.47

5.01

5.79

6.77

8.27

9.12

9.74

LEV

1.00

2.01

3.47

5.01

5.79

6.77

Precision

Accuracy

38.47

25.63

4.13

2.29

2.37

2.15

5.98

1.38

0.96

RTL

[-52.29,91.77]

[-70.26, 45.45]

[-11.21, 4.09]

[-5.50, 3.08]

[-7.33, 1.55]

[-9.78,-1.72]

38.47

30.20

4.13

2.29

2.37

2.15

5.98

1.38

0.96

RIS

74.83

67.38

1.09

0.01

0.03

0.09

1.80

2.22

3.77

4.41

5.28

7.33

8.65

9.04

9.70

LEV

1.80

2.22

3.77

4.41

5.28

7.33

35.94

21.26

7.49

6.25

6.16

2.88

3.20

2.48

2.55

RTL

[-3.8, 131]

[-38.5, 39]

[-8.1, 19.7]

[-20.2, 3.2]

[-23.8, -0.8]

[-4.06, 6.7]

35.94

21.26

7.49

6.25

6.16

2.88

3.20

2.48

2.83

RIS

97.40

82.32

63.44

73.01

87.06

15.22

TABLE II. ICH Q2(R1) VALIDATION CRITERIA OF PLS MODEL IN EACH SOLVENT (A AND B)  $% \mathcal{A}(\mathcal{A})$ 

8.27	[-14.77, 9.36]	6.12	8.65	[-0.1, 12.9]	65.95
9.12	[0.35,5.90]	0.00	9.04	[-3.9, 6.14]	11.98
9.74	[-0.98,2.60]	0.00	9.70	[-4.1, 6.78]	15.56
LI	.OQ U	LOQ	LLC	DQ U	LOQ
3.	187 9	.373	6.56	53 9	.573

\*LEV: Level (mg/mL); MIC: Mean introduced concentration (mg/mL); REB: Relative bias (%); REP: Repeatability (RSD %); INP: Intermediate precision (RSD %); RTL: Relative expectation tolerance limits (%); RIS: Risk (%); ULOQ: Upper LOQ (mg/mL)



Figure 3. Accuracy profile of optimum model in each solvent. The plain line is the relative bias, the dashed lines are the  $\beta$ -expectations tolerance limits ( $\beta$  = 95%) and the dotted lines represent the acceptance limits (±15%). (a) PLS

model for ethanol; (b) PLS model for acetone; (C) PLS model for water; (d) PLS model for DMSO.

solvents. The total errors from water, acetone, and DMSO were significantly larger than the ethanol, indicated that solvent affected prediction error of NIR model.

#### D. Uncertainty assessment

Table III presented several uncertainty results of PLS model in different solvents. The relative expanded uncertainties (REU) with the corresponding introduced concentrations level of each solvent were obtained. To compare the REU, the first concentration level was used as an example. For ethanol, REU was lowest among all solvents, which the value was not above 23.74%. For water, it was 151.10%; for acetone, it was 80.08%; for DMSO, it was 74.81%. It was implied that with a confidence level of 95%, uncertainty results of PLS model changed attributed to different solvents.

 TABLE III.
 ESTIMATES OF MEASUREMENTS UNCERTAINTIES RELATED TO

 THE CITRIC ACID CONCENTRATION IN THE FOUR SOLVENTS AT EACH
 CONCENTRATION LEVEL INVESTIGATED.

	MIC	UB	U	EU	REU
Ethanol	1.12	0.04	0.13	0.27	23.74
	2.03	0.04	0.11	0.23	11.22
	3.20	0.03	0.12	0.24	7.58
	4.40	0.04	0.15	0.29	6.67
	5.57	0.04	0.13	0.26	4.64
	6.77	0.05	0.18	0.35	5.20
	7.97	0.05	0.17	0.34	4.28
	9.36	0.05	0.20	0.39	4.18
	1.12	0.26	0.85	1.69	151.10
	2.03	0.21	0.76	1.53	75.45
	3.20	0.14	0.50	0.99	30.97
itei	4.40	0.14	0.50	1.00	22.72
Ň	5.57	0.08	0.28	0.57	10.18
	6.77	0.08	0.29	0.59	8.71
	7.97	0.16	0.57	1.15	14.39
	9.36	0.15	0.69	1.38	14.76
	0.97	0.11	0.39	0.77	80.08
	1.96	0.20	0.62	1.25	63.87
	3.27	0.03	0.14	0.28	8.57
one	4.42	0.03	0.11	0.21	4.77
seto	5.42	0.04	0.13	0.27	4.93
Ac	6.49	0.04	0.15	0.29	4.48
	7.53	0.16	0.48	0.96	12.69
	8.33	0.04	0.12	0.24	2.92
	9.37	0.03	0.09	0.19	1.99
	1.10	0.11	0.41	0.82	74.81
	2.21	0.12	0.49	0.97	43.92
DMSO	3.57	0.07	0.28	0.55	15.55
	4.82	0.09	0.31	0.63	13.01
	6.02	0.11	0.39	0.77	12.83
	7.23	0.06	0.22	0.43	5.99
	8.13	0.09	0.28	0.55	6.79
	8.94	0.08	0.23	0.47	5.26
	9.57	0.09	0.29	0.57	5.97

<sup>\*</sup> Mean introduced concentration (MIC, mg/mL); Uncertainty of the bias (UB, mg/mL); Uncertainty (U, mg/mL); Expanded uncertainty (EU, mg/mL); Relative expanded uncertainty (REU, %).

#### **IV. CONCLUSIONS**

Our study confirmed that solvent had great effect on the LLOQ of NIR. Based on the optimum combinational pretreatments method, PLS model enable to quantify citric acid

in each solvent were developed. Each model was successfully validated based on accuracy profile. The results demonstrated that ethanol was the best quantitative solvent for citric acid, and the LLOQ was 2.791 mg/mL. In water, acetone and DMSO, the LLOQ was 4.542 mg/mL, 3.187 mg/mL and 6.563 mg/mL, respectively. This paper was to investigate the LLOQ of NIR for citric acid in different solvent system (ethanol, water, acetone, DMSO). We have to point out that LLOQ of NIR in different solvents was approximate value in this study. However, this problem could be solved if we consider obtaining optimum model such as iPLS, MWPLS, CARS.

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