

Raman Chemical Imaging and Chemometrics as Tools for Investigation of Counterfeit Products

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INTRODUCTION The pharmaceutical counterfeiting cause increasing problems on the pharmaceutical market [1]. Illegal or counterfeit drugs are manufactured under the full exclusion of GMP regulations. These may contain unknown impurities and lower amount of active ingredients typically. The counterfeit products may cause ineffectiveness or have low to no bioavailability and even serious side effects.

Vibrational spectroscopic techniques such as Raman or near-irfrared combined chemometrics are used in many aspects of the pharmaceutical industry [2,3] and are applied for fake drug analysis [4,5]. Some kind of classification or pattern recognition methods are applied in most of cases to distinguish counterfeit and genuine products [6,7]. The chemical imaging technique can be utilized to obtain spatial distribution of unknown components using multivariate curve resolution or factor analysis methods [8]. Hence, besides extracting qualitative composition without any apriori information, these combined tools have addition benefit in exploration of manufacturing technologies.



Model set 1: model tablets contained 10% imipramine as active ingredient (API), 76% microcrystalline cellulose (MCC), 10% maize starch, 3% hydroxypropyl-methyl-cellulose (HPMC) and 1% magnesium stearate. Manufacturing technologies: ‘D’ tablets - dry homogenization and direct compaction (Fette EX1 eccentric press), ‘HS’ tablets - wet high-shear granulation (ProCepT 4M8 high shear granulator) before compaction, ‘F’ tablets - fluidized bed granulation (Glatt GCPG-1 fluidized bed granulator).

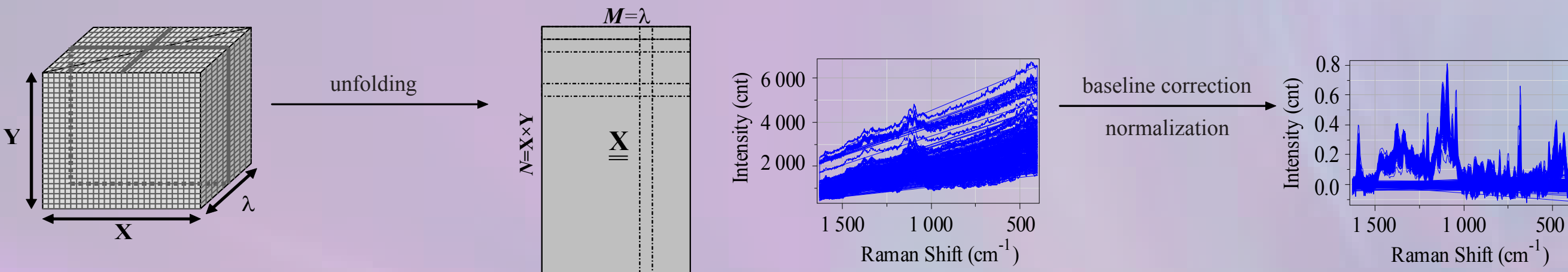
Model set 2: 3.75% API, 57.5% lactose-monohydrate, 37,5% maize starch and 1.25% magnesium stearate were compacted in tablet form led to heterogeneous distribution

Analysis Horiba Jobin-Yvon LabRAM system. Coupled with an external 785 nm diode (for Model 1), 532 nm Nd-YAG (for Model 2) laser source and an Olympus BX-40 optical microscope. Objectives of 10x and 100x magnification were used for optical imaging and spectrum acquisition. Spectral range: 400-1630 cm⁻¹ and 310-1760 cm⁻¹. Mapping step size: 2 μm (100x) or 10 μm (10x) along both the X and Y coordinates.

Data evaluation: Matlab 7.6, PLS_Toolbox 6.0.1, MIA_Toolbox 2.0.1, LabSpec 4.02.

Preprocessing and data analysis

Reference: Classical Least Squares (CLS) with pure component spectra

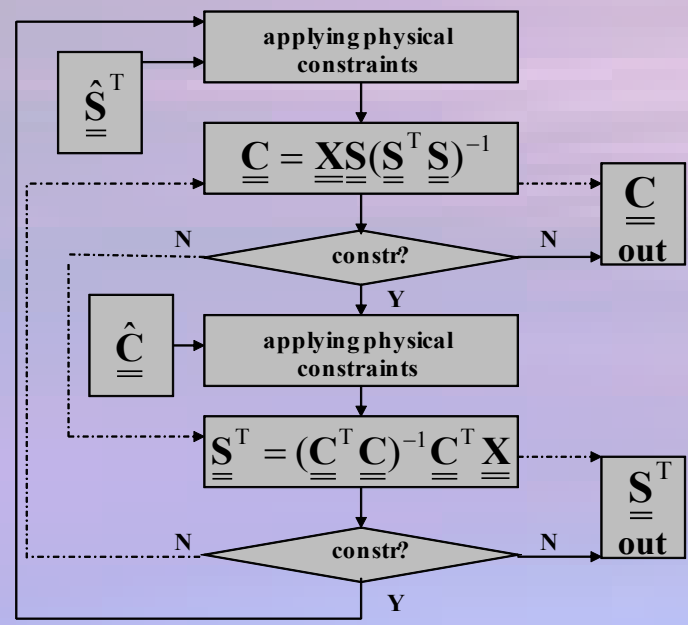


- Four types of curve resolution methods

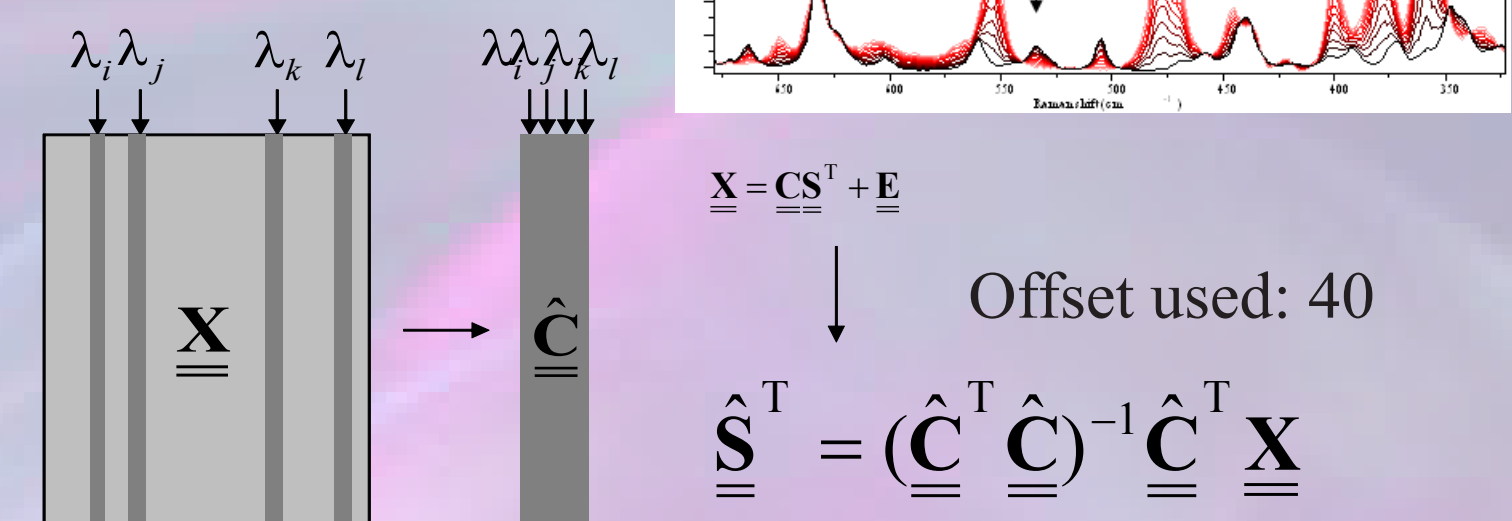
Multivariate curve resolution - alternate least squares (MCR-ALS) [9]

Initialization: SMMA

- Applied constraints:
- (1) non-negativity of concentrations
 - (2) non-negativity of spectra
 - (3) closure
 - (4) contrast (or angle)



Self-modeling mixture analysis (SMMA) [10]

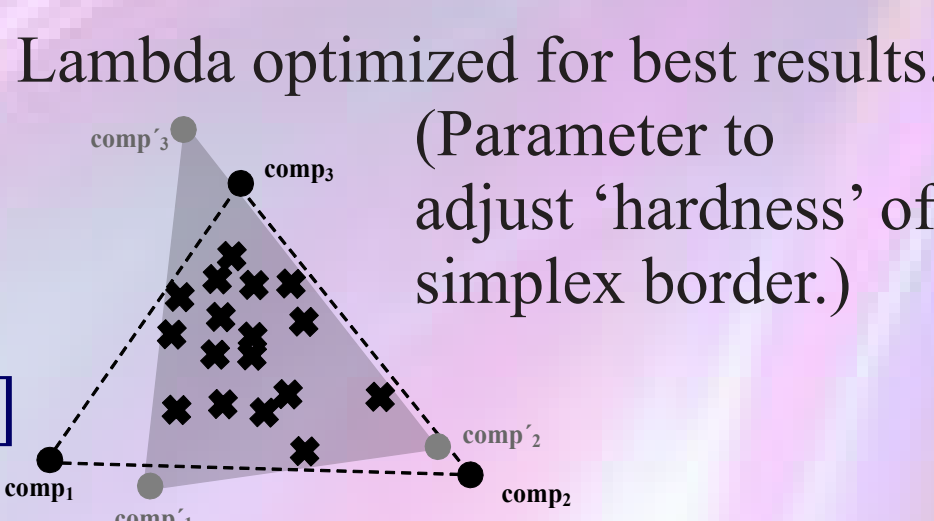


Positive matrix factorization [11]

$$\mathbf{E} = \mathbf{X} - \mathbf{CS}^T$$
$$Q_{\text{mod}} = \alpha \alpha^T \left(\frac{\alpha(\mathbf{X} - \mathbf{CS}^T)}{\sigma_{ij}} \right)^2 - \alpha \alpha^T \log C_{ik} - \beta \alpha \alpha^T \log S_{ij} + \gamma \alpha \alpha^T C_{ik}^2 + \delta \alpha \alpha^T (S_{ij}^T)$$

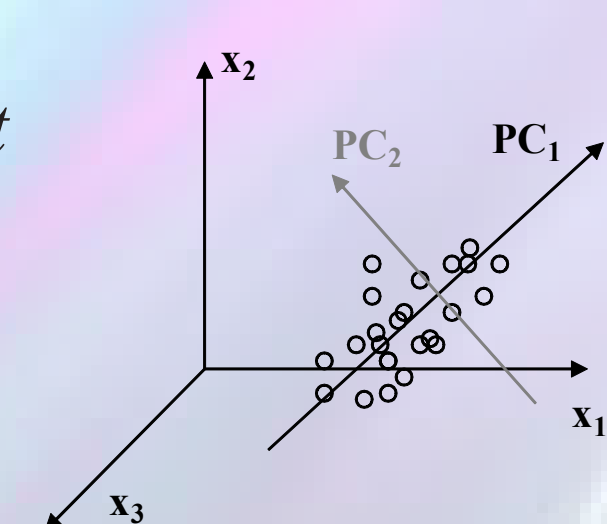
Various values tried for every parameter. Rotational parameter (peak) optimized [9].

Simplex Identification via Split Augmented Lagrangian [12]



- The most common method based on factor analysis

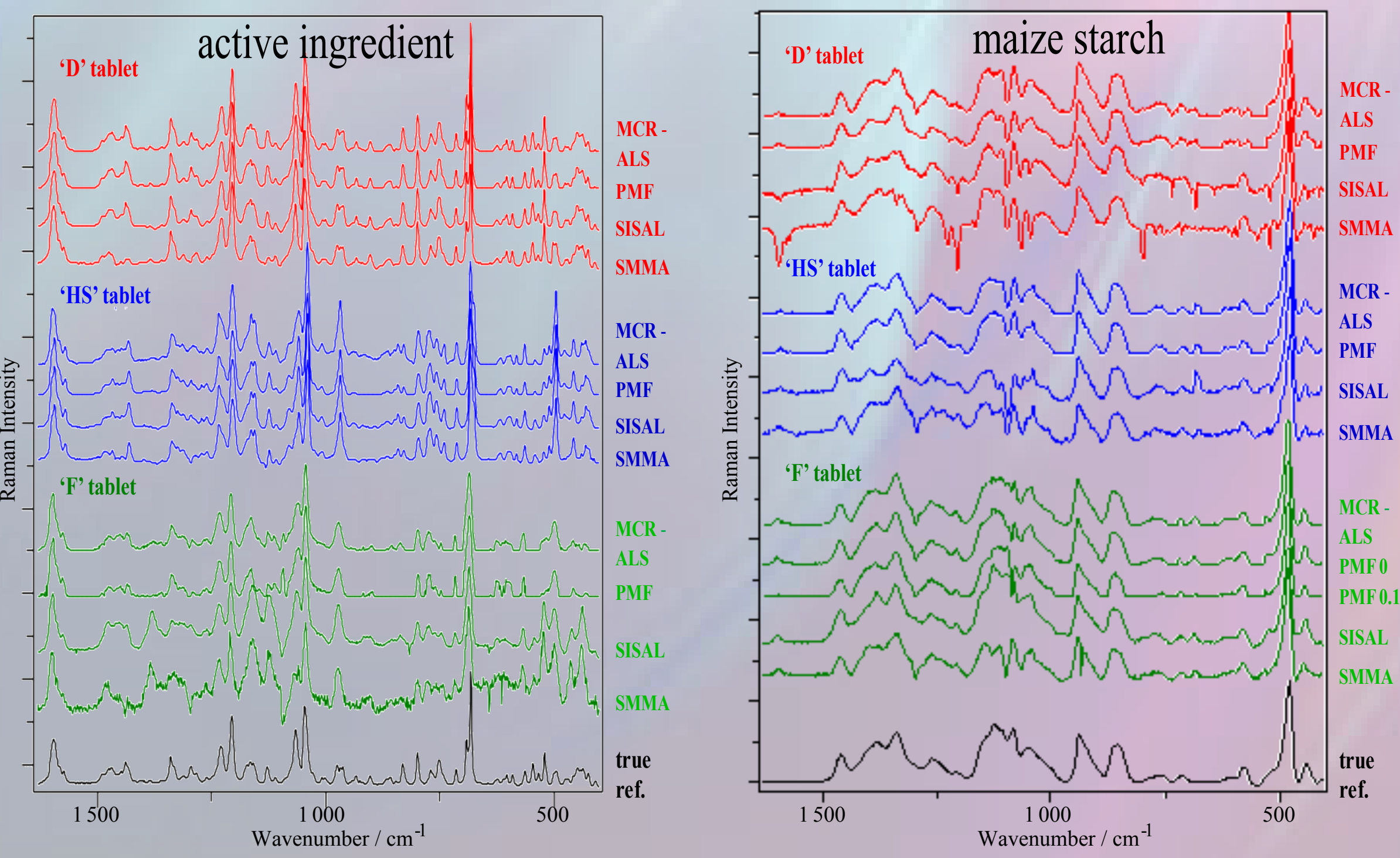
Principal Component Analysis (PCA) [13]



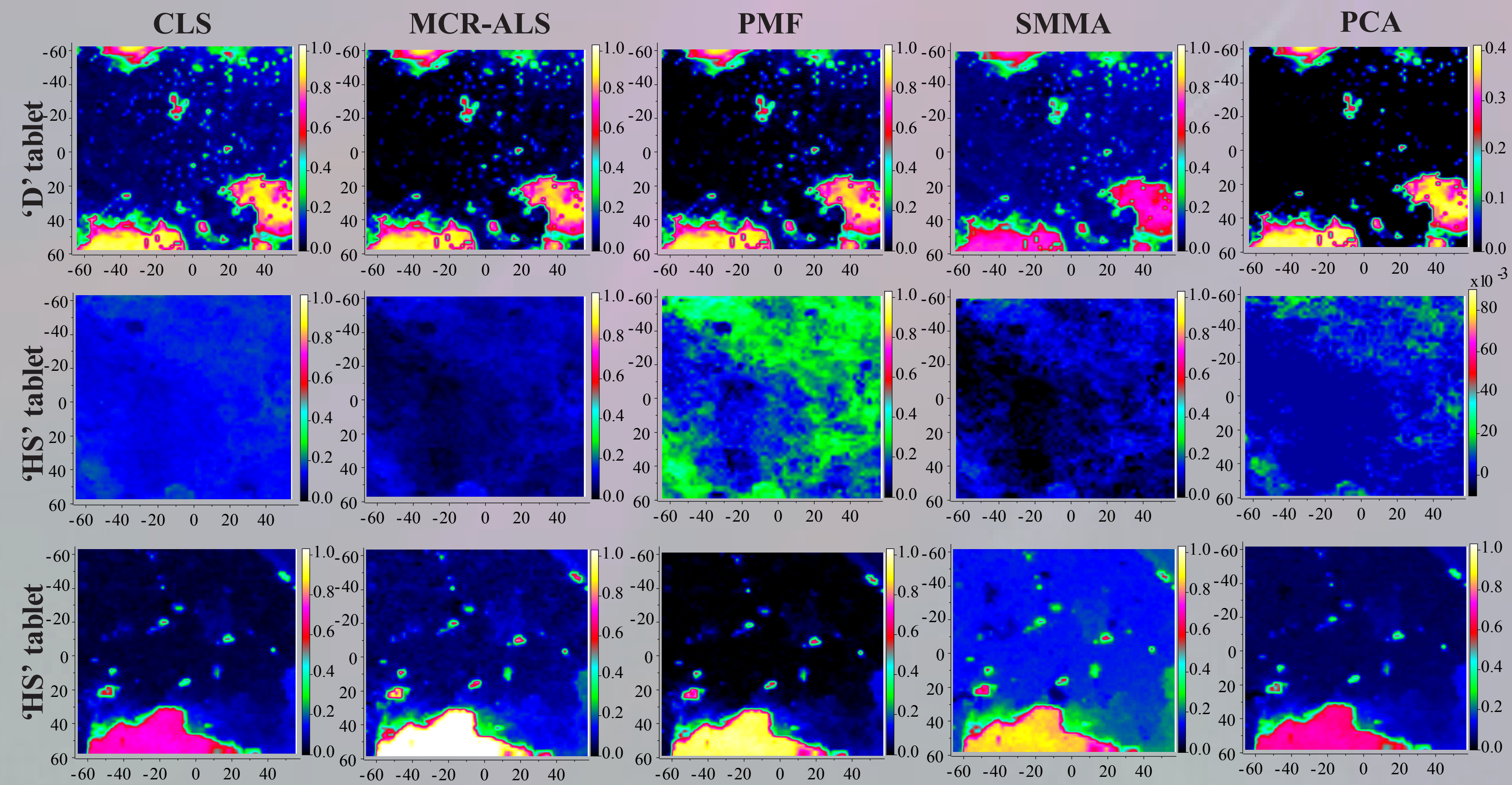
$$\mathbf{X} = \mathbf{U}\mathbf{S}\mathbf{V}^T$$
$$\mathbf{X} = \mathbf{CS}^T + \mathbf{E}$$
$$\hat{\mathbf{S}}^T = \mathbf{V}^T$$
$$\hat{\mathbf{C}} = \mathbf{U}\mathbf{S}$$

Assumption: the PCs of the Raman image correspond to the spectra of the pure components.

Tablet characterization with different manufacturing process (Model 1)



It can be seen on the examples of API and maize starch resolved spectra. MCR-ALS and PMF are both able to give very accurate estimations for the pure component spectra with high correlation and high fit, but only MCR-ALS gives high quality loadings for all components in one calculation, if the concentration of a certain component (the API in this study) is almost constant. SISAL and SMMA also provided fairly good pure component spectra, but their performance is lower than the two former techniques. All five components including HPMC spectra are detected only from the Raman map of 'D' tablet with MCR-ALS and PMF due to its heterogeneous distribution.

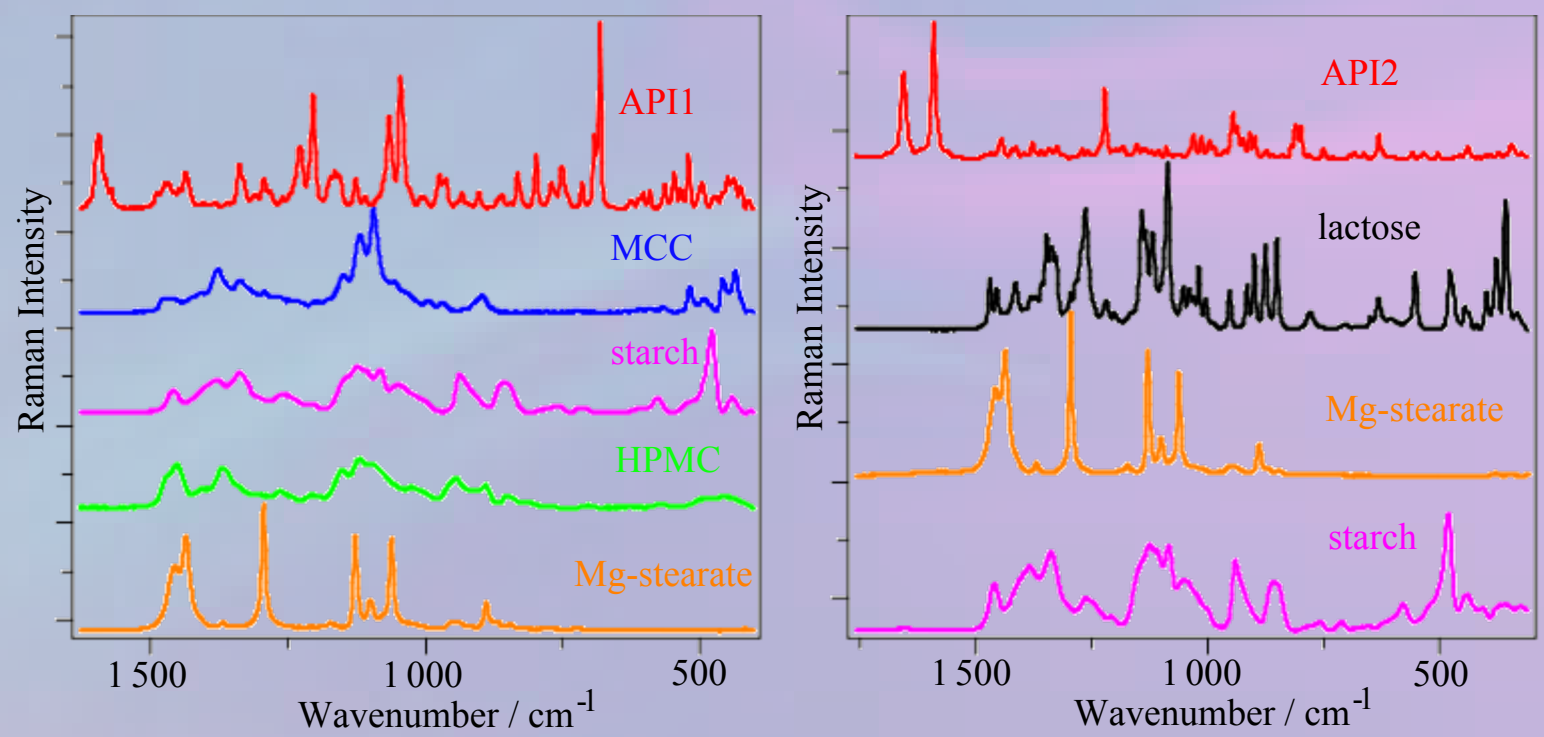


Noise level	PCA	SMMA	MCR-ALS	PMF	True number of components
~1%	4	4	5	4	5
10%	4	4	4	4	
30%	3	3	4	4	
50%	3	2	4	4	

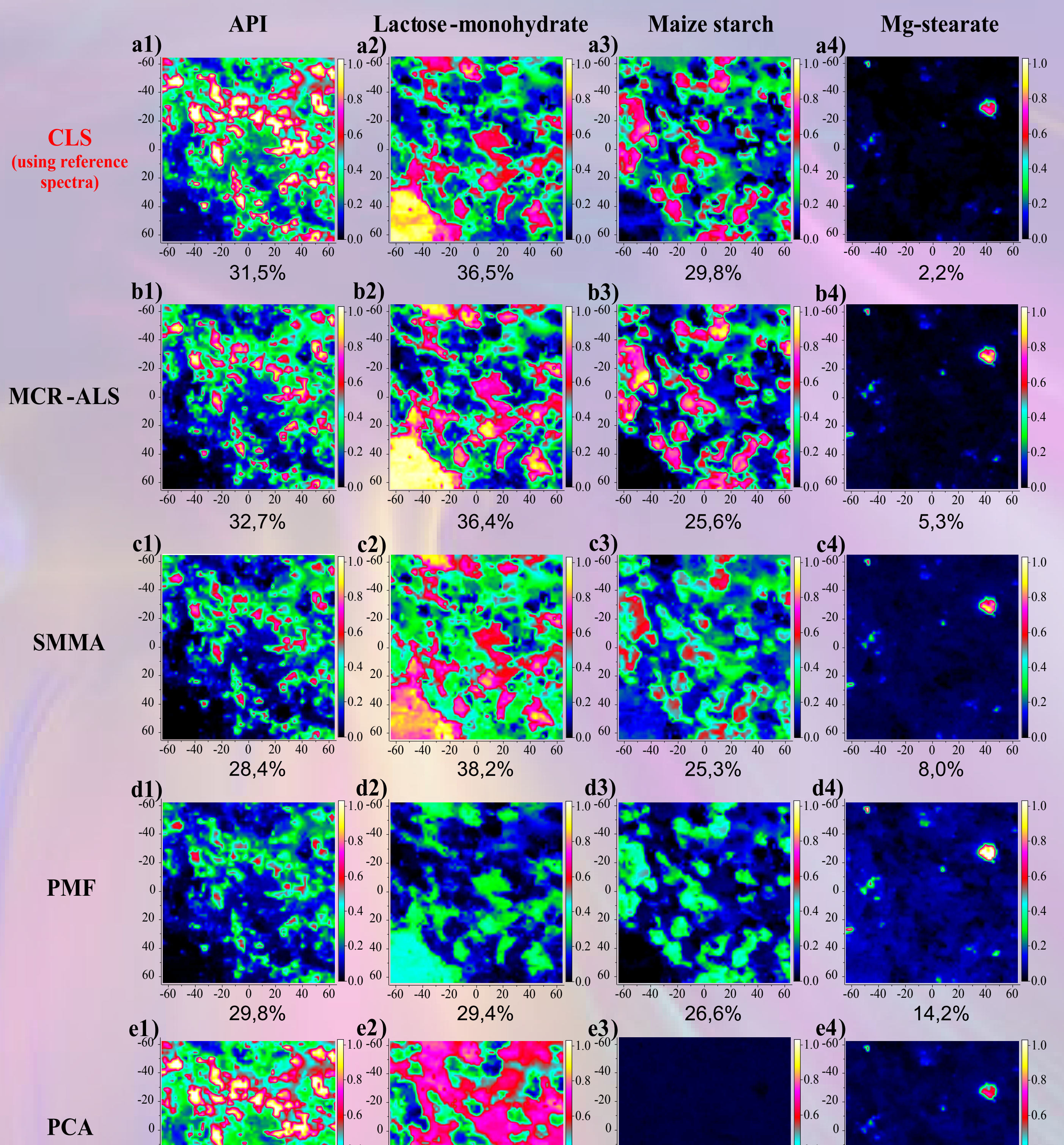
Number of extracted components with different noise level on 'D' tablet

Effect of noise for the number of extracted components

The preprocessed spectra have also loaded with different noise level to investigate whether methods can better handled the deteriorating spectrum quality. Adding 10-50% homoscedastic noise the efficiency of PCA and SMMA were worsened. The number of determined components decreased in these cases.



Quantification of tablet components (Model 2)



Distribution maps for each methods were obtained using the resolved spectra in the model. Spectral concentrations were calculated according to the classical least squares modelling and the mean values indicated below plotted maps. The calculations served estimated concentration. It can be more accurate building some calibration models with known concentration levels from API.

The spectral concentrations can be compared with each other assumed the reference values of the calculations from the known reference spectra. Therefore there is no difficulty to compare counterfeit tablets distribution maps to the genuine products. The closest values to the references were obtained with MCR-ALS.

CONCLUSIONS

Counterfeiting tablets can differ from the original one by the content or quality of their ingredients. Furthermore the fluctuation of the quality of counterfeit products is generally larger than that of the original ones highlighting the role of statistics in the identification. Combination of Raman-microspectrometry and chemomerty provided several non-destructive tools for separating the two types of products from each other. The pattern recognition methods such as PCA or the more advanced supervised classification methods such as PLS-DA and SVM can be efficient for giving simple yes or no answers. However, the real advantages of Raman chemical mapping can be utilized also for giving estimations even if no a-priori information is available regarding the composition. In such cases the self-modelling curve resolution, methods, such as MCR-ALS, PMF, SISAL, SMMA can be used. Not only the main components but also the impurities (e.g. polymorphs) can be determined this way. The identification of the applied technology and the stable or fluctuating quality show not only the differences but also the source of differences between products. Quantitative analysis is also feasible but accurate result requires calibration.

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