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Research paper

Choice and validation of a near infrared spectroscopic application for the identity control of starting materials. Practical experience with the EU draft Note for Guidance on the use of near infrared spectroscopy by the pharmaceutical industry and the data to be forwarded in part II of the dossier for a marketing authorization

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Abstract

Recently the CPMP/CVMP sent out for consultation the draft Note for Guidance (dNfG) on the use of near infrared spectroscopy (NIRS) by the pharmaceutical industry and the data to be forwarded in part II of the dossier for a marketing authorization. We explored the practicability of this dNfG with respect to the verification of the correct identity of starting materials in a generic tablet-manufacturing site. Within the boundaries of the dNfG, a release procedure was developed for 12 substances containing structurally related compounds and substances differing only in particle size. For the method development literature data were also taken into consideration. Good results were obtained with wavelength correlation (WC), applied on raw spectra or second derivative spectra both without smoothing. The defined threshold of 0.98 for raw spectra differentiated between all molecular structures. Both methods were found to be robust over a period of 1 year. For the differentiation between the different particle sizes a subsequent second chemometric technique had to be used. Soft independent modelling of class analogy (SIMCA) with a probability level of 0.01 proved suitable. Internal and external validation I according to the dNfG showed no incorrect rejections or false acceptances. External validation II according to the dNfG was carried out with 95 potentially interfering substances from which 46 were tested experimentally. Macrogol 400 was not distinguished from macrogol 300. For the complete verification of the identity of macrogol 300 test A of the European Pharmacopoeia is needed in addition to the NIRS application. A release procedure developed with WC applied on raw spectra and SIMCA as a second method, which is different from the preferred method of the dNfG, was tested in practice with good results. We conclude that the dNfG has good practicability and that deviations from the preferred methods of the dNfG can also give good differentiation.

Keywords: Near infrared spectroscopy; Identification; Starting materials; Release testing

1. Introduction

The correct identity is the most important quality aspect of substances to be used for the manufacture of medicinal products. The correct identity of a supply of starting materials can only be guaranteed if individual samples are taken from all the containers and the identity of each sample is tested [1]. Substances described in the European Pharmacopoeia (EP) should always meet these pharmacopoeial specifications [1]. So, it is clear that for the verification of the identity, every container of a batch of starting material must be subjected to all EP identification tests. However, according to the general notices of the EP, "with the agreement of the competent authority, alternative methods of analysis may be used" [2]. Near infrared spectroscopy (NIRS) combined with chemometric algorithms for spectrum comparison is one of the most attractive

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alternative analytical methods for identity control of starting materials. NIRS offers high discriminatory power, fast analysis, no or very little sample preparation and is non-destructive. In addition, NIRS offers in many cases the possibility for the verification not only of the chemical structure of the starting material, but also for the verification of the correct particle size, polymorphic form, water content and other physical attributes. In 1997, a monograph on NIRS was included in the EP [3]. This monograph leaves substantial freedom in the technical details of the NIRS methodology to be used. In November 2001, the CPMP/CVMP sent out for consultation a draft Note for Guidance (dNfG) on the use of NIRS by the pharmaceutical industry and the data to be forwarded in part II of the dossier for a marketing authorization [4]. This dNfG provides more detailed recommendations on wavelength selection, pre-treatment of the spectra, chemometric classification algorithms, suitable threshold(s) and the validation procedure. Recently, a finalized version of the Note for Guidance on NIRS has been adopted by the CPMP and published [5]. Concerning the identification and qualification of pharmaceutical substances, the guidance in the finalized version is similar to the draft, however some explanation has been provided. Also a new monograph is submitted for comments in Pharmeuropa 15.1 [6]. In this monograph information on possibly suitable algorithms and pre-treatments has been added. This information is in line with the CPMP Note for Guidance on NIRS, however, preferred methods are not indicated in the revised monograph. It contains appropriate guidance on the validation of methods for qualitative analysis, very much in line with the CPMP Note for Guidance.

The objectives of this study were not to justify or explain the contents of the dNfG but to examine and demonstrate its practicability. In the present study we tested the practicability of the recommendations of the dNfG for the verification of the identity of incoming starting materials in a production site of generic tablets. From approximately 1000 starting materials, including packaging materials, we selected 12 substances that we expected to be the most challenging for setting up a release procedure by NIRS. These 12 substances are shown in Table 1.

For the method development, the main decisions to be taken are the choice of the wavelength range, the spectral pre-treatment (if any), the chemometric technique for spectra comparison and its thresholds. According to the dNfG the chemometric technique wavelength correlation (WC) of the second derivative spectra and maximum wavelength distance (MWD) are preferred. 'Smoothing' may be used as additional pre-treatment. The use of other combinations or other methods of pre-treatment should be justified.

We compared these recommendations of the dNfG with the results of a literature survey. These results are compiled in Table 2. The literature reports a large variability in combinations of spectrum pre-treatments and chemometric algorithms. Good results are indeed described for WC on derivative spectra [7–9]. However, good results are also reported with principal components analysis (PCA) on derivative spectra [7,10–15]. MWD with pre-treatments other than derivatives were used with good results [7,10,11,16]. Other workers reported good results with soft independent modelling of class analogy (SIMCA) or with spectral match value and conformity index [17,18].

In view of this wide variability of techniques used we selected two different approaches to develop a NIR method for identity control of starting materials. As the NfG leaves open the possibility to make other choices we tested WC on the raw spectra and SIMCA as a second chemometric technique. To be able to compare the results we also investigated WC on the second derivative spectra, as the preferred method in the dNfG. We did not explore the possibilities of MWD as a second technique because this software was not available.

Table 1
Substances for which the release procedure based on NIRS was developed, the number of different batches used for calibration and the number of different batches used for external validation I

Substance	Number of batches used for calibration	Number of batches used for external validation I
Precirol®*	8	2
Lubritab®#	5	1
Cortisone acetate	8	2
Prednisone	8	2
Paracetamol 180 μm	5	1
Paracetamol crystalline	8	2
Paracetamol 45 μm	3	1
Tolbutamide	8	2
Furosemide	8	2
Prednisolone	8	2
Glycerol 85%	8	2
Macrogol 300	8	2

* Atomized glycerol palmitostearate, made of mono-, di- and triglycerides of saturated fatty acids; # made from fully hydrogenated refined cottonseed oil.

2. Materials and methods

2.1. General set-up

The study was carried out with the co-operation of Magnafarma, a manufacturer of generic tablets located in Zaandam, The Netherlands. In our laboratory we developed an independent release procedure for the 12 selected substances with NIRS, using samples provided by Magnafarma. All these samples were released prior to shipping to our laboratory by Magnafarma using the identity tests of the EP. Our NIRS-release procedure was

Table 2
NIRS methods used for the verification of identity of pharmaceutical materials

Study	Substances	Spectrum pre-treatment	Chemometric Technique	Results
Gerhäusser et al. [7]	Benzodiazepines	No pre-treatment, second derivative, wavelength selection	Wavelength correlation, maximum wave distance, principal components analysis	Second derivative with principal components analysis and wavelength correlation gave best results
Ulmschneider et al. [8]	Active substances	Second derivative	Wavelength correlation	Unequivocal identification
Yoon et al. [9]	Solvents	Second derivative	Wavelength correlation	Unequivocal identification
Candolfi et al. [10]	Excipients	Baseline detrending and offset, multiple scatter correction, second derivative	Principal components analysis, maximum wave distance, triangular potential function	Standard normal variate transformation with maximum wave distance gave best results
Candolfi et al. [11]	Cellulose microcryst	Standard normal variate transformation	Principal components analysis, maximum wave distance	Updating with maximum wave distance more straightforward than with principal components analysis
Ulmschneider et al. [12]	Intermediates and actives	First derivative, wavelength selection	Principal components analysis, cluster analysis module from Buchi	Unequivocal identification
Ulmschneider et al. [13]	Active substances	First derivative	Principal components analysis	Unequivocal identification
Ulmschneider et al. [14]	Starches, sugars and celluloses	Normalization by closure and first derivative, multiple scatter correction and second derivative	Principal components analysis, cluster calibration module from Buhler	Unequivocal identification
Krämer et al. [15]	Celluloses and cellulose ethers	Multiple scatter correction, first derivative, wavelength selection	Principal components analysis versus soft independent modelling of class analogy	Additional tests are required for some identifications
Gemperline et al. [16]	Excipients including celluloses	No pre-treatment	Maximum wave distance, soft independent modelling of class analogy, Mahalanobis distance	Maximum wave distance suitable
Plugge et al. [18]	Ampicillin trihydrate and celluloses		Spectral match value, conformity index	Accepted as in-house test by the FDA

operated independently from the routine release procedure of Magnafarma and the results of our release procedure were not used for the decisions of Magnafarma.

2.2. NIRS analyses

NIR-spectra were recorded on a Spectrum Identicheck FT-NIR system (Perkin-Elmer Ltd., Beacconsfield Bucks, UK) with an IdentiCheck Reflectance Accessory (ICRA) with the standard Spectrum Identicheck software, version 2.00 and Quant + software, version 4.10 including WC and SIMCA to acquire and to process the data. WC was always applied using the default filter setting which includes a resolution filter. Measurements were carried out with an optical resolution of 16 cm^{-1} over the spectral range 12000–3000 cm^{-1} and 64 scans were co-added. A PbS detector was used. Spectralon was used as a background reference for solid samples and a Petri dish with a reflector was used for liquids. Solid samples (1 g) were measured as delivered in a 4 ml glass vial (Alltech) with closure and spectra were recorded in the diffuse reflection mode. Liquid samples were poured into a Petri dish with a metal reflector (Perkin-Elmer) and the spectra were recorded in the transreflection mode.

2.3. Samples

Samples were taken by Magnafarma and shipped in the 4 ml glass vials with screw cap. They were stored in the dark at ambient room temperature and humidity.

2.4. Method development

2.4.1. Calibration

A reference library was constructed with NIR-spectra of the 12 pharmaceutical substances. The number of calibration batches per substance is shown in Table 1. From each calibration batch, one spectrum was recorded, with the exception of the paracetamol calibration batches (see below). All NIR-spectra were recorded on 1 day in a row. The same raw data were used to construct a library of second derivative spectra.

2.4.2. Internal validation and threshold setting

Internal validation was carried out with the calibration spectra obtained. Three NIRS methods were investigated on their power to separate the 12 substances:

- WC on the raw spectra over the spectral range 10000–4000 cm^{-1}, threshold 0.95.

Table 3
Substances considered for external validation II and justification for exclusion from experimental validation

Potential interfering substance	Experimental validation	Justification for absence of experimental validation
4-Aminopyridine	Yes	
4-Aminosalicylic acid micr	Yes	
Acetylsalicylic acid <180 μm	Yes	
Aminophenazone	No	Structure; very different spectrum expected
Beclometasone	No	Number of steroids already included
Beclometasone dipropionate	Yes	
Benorilate	No	Not available: presence at site unlikely
Benzoic acid	Yes	
Betametasone dipropionate	Yes	
Betametasone	Yes	
Betametasone valerate	Yes	
Bumetanide	Yes	
Carbasalate calcium	No	Acid already included
Cetiol V	Yes	
Cetylstearyl alcohol	Yes	
Chlorbutanole	No	Powder; very different spectrum expected
Clobetasol dipropionate	No	Number of steroids already included
Clobetasone butyrate micr	Yes	
Cortisone	Yes	
Deoxycortone	Yes	
Dexametasone disodium phosphate	No	Number of steroids already included
Dexametasone micr	Yes	
Dimethicone	No	Structure; very different spectrum expected
DMSO	No	Structure; very different spectrum expected
Etacrynic acid	No	Not available
Ethinyl estradiol	Yes	
Ethyl glycol	Yes	
Fludrocortisone acetate	Yes	
Flumetasone pivalate micr	Yes	
Fluocinolone acetonide	No	Number of steroids already included
Fucidic acid	No	Structure; very different spectrum expected
Furadantine	No	Typical colour: structure; very different spectrum expected
Furazosine (prazosine)	No	Structure; very different spectrum expected
Fusafungine	No	Not available: structure (polypeptide); very different spectrum expected
Glibenclamide	Yes	
Gliclazide	Yes	
Glipizide	No	Not available
Glycopyrrolate	No	Powder; very different spectrum expected
Glycerol	Yes	
Glycerol diacetate	No	Not available: not likely to be present
Glycerol dichlorhydrine	No	Not available: not likely to be present
Glycerol dimethylketal	No	Not available
Glycerol formal	No	Not available
Glycerol monostearate 46-54	Yes	
Glycerol triacetate	No	Not available
Glyceryl aminobenzoate	No	Semisolid; very different spectrum expected
Glyceryl guaicolate	No	Powder; very different spectrum expected
Glyceryl monoacetate	No	Not available: not likely to be present
Glyceryl tolyl ether	No	Powder; very different spectrum expected
Glycine	No	Powder; very different spectrum expected
Glycol salicylate	No	Not available
Hydrocortisone micr	Yes	
Hydrocortisone acetate micr	Yes	
Ibuprofen	Yes	
Macrogol 400	Yes	
Metformine HCl	No	Not available
Methyl prednisolone	Yes	
O-Toluamide	No	Explosive; not available
Papaverine HCl	No	Structure; very different spectrum expected
Para-aminobenzoic acid	No	Not available
Paracetaldehyde	No	Liquid; very different spectrum expected
Paracetamol 500-90	Yes	

(continued on next page)

Table 3 (*continued*)

Potential interfering substance	Experimental validation	Justification for absence of experimental validation
Parachloramine (meclozine HCl)	No	Structure; very different spectrum expected
Parachlorphenol	No	Structure; very different spectrum expected
Paracortol	Yes	Is prednisolone
Paradichlorbenzene	No	Structure; very different spectrum expected
Paraffin	Yes	
Paraformaldehyde	No	Structure; very different spectrum expected
Paramethadione	No	Liquid; very different spectrum expected
Paramethasone	No	Number of steroids already included
Paraoxon	No	Liquid; very different spectrum expected
Parathion	No	Liquid; very different spectrum expected
Phenacetine	Yes	
Phenazone	No	Structure; very different spectrum expected: not available
Polysorbate 80	Yes	
Prednisolone acetate	Yes	
Prednisolone hemisuccinate	Yes	
Prednisolone metasulphobenzoas	No	Number of steroids already included
Prednisolone sodium phosphate	Yes	
Progesterone	Yes	
Propyfenazone	No	Structure; very different spectrum expected: not available
Propylene glycol	Yes	
Salicylic acid <90	Yes	
Sorbitol apyrogene	Yes	
Stearic acid pulverized	Yes	
Testosterone propionate	Yes	
Testosterone micr	No	Number of steroids already included
Tolazamide	Yes	
Tolazoline	No	Structure; very different spectrum expected: not available
Tolazoline HCl	No	Structure; very different spectrum expected: not available
Tolbutamide sodium	No	Not available
Tolcyclamide	No	Not available
Tolfenamic acid	No	Structure; very different spectrum expected
Tolmetine	No	Not available

- WC on the second derivative spectra over the spectral range 10000–4000 cm^{-1}, threshold 0.95.
- For the paracetamol substances, additional SIMCA on second derivative spectra in the spectral range 10000–4000 cm^{-1} was investigated. Multiple scatter correction (MSC) over the total spectral range was applied. Wavelength selection was used skipping the part 5500–5100 cm^{-1} to correct for water vapour.

To obtain sufficient data for paracetamol using this technique, two spectra were recorded for each calibration batch. The two spectra were collected on different days.

2.4.3. Tentative release procedure

After internal validation, the tentative release procedure developed was:

- Precirol® and Lubritab®: WC with a threshold of 0.98 on the raw spectra, spectral range 10000–4000 cm^{-1}. For all other substances WC with a threshold of 0.95 applied on raw spectra in the frequency range 10000–4000 cm^{-1}.
- Prednisone, prednisolone, cortisone acetate, furosemide, tolbutamide, macrogol 300 and glycerol 85%: WC with a threshold of 0.95 on second derivative spectra, spectral range 7000–4000 cm^{-1}.
- Paracetamol 45 μm, 180 μm and crystalline: WC with a threshold of 0.95 on second derivative spectra, spectral range 7000–4000 cm^{-1} and subsequent use of SIMCA with a probability level of 0.01 on second derivative spectra, range 10000–4000 cm^{-1} with MSC and a correction for water vapour.

2.4.4. External validation I

This tentative release procedure was challenged with independent batches of the 12 substances. These batches were not previously used for the construction of the reference library. The number of batches used for the external validation I pro-substance is shown in Table 1.

2.4.5. External validation II

Name and structural analogues and the other substances in use by Magnafarma were considered for external validation II (see Table 3). From 95 considered substances, 46 were actually experimentally used to challenge the tentative release procedure (again see Table 3).

2.5. Robustness

The robustness of the NIRS part of the procedure was tested on Precirol® and Lubritab®. Spectra of ten batches of Precirol® and six batches of Lubritab® were recorded five times over a period of 12 months. PCA was carried out on the second derivative spectra and 95% confidence intervals were calculated for each series of plots.

A library of raw spectra, spectral range 10000–4000 cm^{-1}, was constructed of two spectra of Precirol® and one spectrum of Lubritab® from each of the five series of recorded spectra. Mean correlation coefficients, standard deviation and range were calculated on all remaining spectra. The same procedure was carried out on the second derivative spectra, spectral range 7000–4000 cm^{-1}.

2.6. Final release procedure

The final release procedure chosen was:

- Precirol® and Lubritab®, prednisone, prednisolone, cortisone acetate, furosemide, tolbutamide, glycerol 85%: WC with a threshold of 0.98 of the raw spectra, spectral range 10000–4000 cm^{-1}.
- Paracetamol 45 μm, 180 μm and crystalline: WC with a threshold of 0.98 of the raw spectra, spectral range 10000–4000 cm^{-1} and subsequent SIMCA with a probability level of 0.01 on second derivative spectra, spectral range 10000–4000 cm^{-1}.
- Macrogol 300: WC with a threshold of 0.98 on the raw spectra, spectral range 10000–4000 cm^{-1}, and subsequent identity test A of the EP.

2.7. The final release procedure in practice

The final release procedure was challenged in practice in two runs. The first run included 12 samples containing both correctly labelled and incorrectly labelled compounds. The second run included 12 coded samples without a labelled identity.

3. Results and discussion

The substances included in this study were chosen from the approximately 1000 starting materials, including packaging materials, in use by Magnafarma. Prednisone, prednisolone and cortisone acetate were chosen because of their chemical similarity. Furosemide and tolbutamide were chosen as representatives of common pharmaceutical substances. Paracetamol 45 μm, 180 μm and crystalline were chosen to explore the possibilities to differentiate between different particle sizes of substances having the same molecular structure. Precirol® (atomized glycerol palmitostearate, made of mono-, di- and triglycerides of saturated fatty acids) and Lubritab® (made from fully hydrogenated refined cottonseed oil) were suggested by Magnafarma who had experienced difficulties in discriminating between these two substances. Indeed, these two substances gave very similar NIR-spectra (see Fig. 1). Glycerol 85% and macrogol 300 were included in the study to explore the practicability of the recommendations of the dNfG for liquids.

The results of the calibration and the internal validation are shown in Table 4. The distance is a standard for the discriminating power between two substances and is the difference between the correlation coefficients of

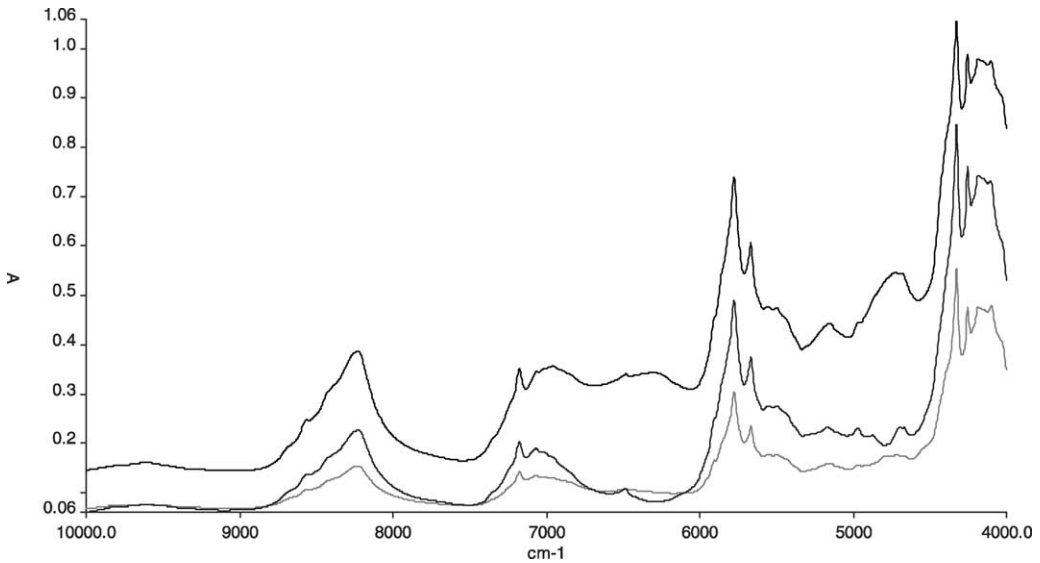

Fig. 1. NIR-spectra of Precirol® (lower), Lubritab® (middle) and glycerolmonostearate (upper).

Table 4
Results of internal validation with several WC techniques applied to the reference library

	WC of raw spectra (10000–4000 cm^{-1})				WC of second-derivative spectra (10000–4000 cm^{-1})				WC of second-derivative spectra (7000–4000 cm^{-1})			
	C_m	S	S_d	D	C_m	S	S_d	D	C_m	S	S_d	D
Precirol®	0.9993	0.0010	0.0004	0.02	0.9865	0.0083	0.0034	0.00	0.9937	0.0099	0.0031	0.10
Lubritab®	0.9996	0.0007	0.0003	0.02	0.9907	0.0049	0.0023	0.00	0.9990	0.0002	0.0001	0.09
Cortisone acetate	0.9987	0.0061	0.0020	0.34	0.9788	0.0197	0.0063	0.76				
Prednisone	0.9995	0.0011	0.0004	0.32	0.9831	0.0070	0.0021	0.76				
Paracetamol 180 μm	0.9999	0.0001	0.0001	0.00	0.9935	0.0031	0.0013	0.01	0.9997	0.0002	0.0001	0.00
Paracetamol crystalline	0.9990	0.0017	0.0006	0.05	0.9940	0.0029	0.0011	0.01	0.9995	0.0003	0.0001	0.00
Paracetamol 45 μm	1.0000	0.0001	0.0001	0.00	0.9904	0.0016	0.0009	0.00	0.9996	0.0002	0.0001	0.00
Tolbutamide	1.0000	0.0001	0.0001	0.61	0.9971	0.0009	0.0003	0.73				
Furosemide	0.9999	0.0001	0.0001	0.79	0.9919	0.0025	0.0009	0.82				
Prednisolone	0.9997	0.0009	0.0003	0.53	0.9876	0.0061	0.0021	0.87				
Glycerol 85%	0.9999	0.0012	0.0004	0.38	0.8855	0.0160	0.0052	0.58	0.9912	0.0060	0.0018	0.71
Macrogol 300	0.9985	0.0084	0.0029	0.42	0.9053	0.0236	0.0089	0.60	0.9891	0.0111	0.0041	0.71

C_m, mean correlation; S, range of correlation coefficients; S_d, standard deviation of correlation coefficients; D, distance = the difference in correlation coefficients from the correct identified substance and the first incorrect identification.

the correctly identified substance and the first incorrect identification. For most of the differentiations, both WC on raw spectra and WC applied on second derivative spectra gave a sufficiently high distance value. However, for the crucial discrimination between Precirol® and Lubritab® WC using second derivative spectra as suggested by the dNfG did not differentiate between these two substances, as the distance was zero. When the frequency range of the second derivative spectra was narrowed to 7000–4000 cm^{-1}, the distance values increased to 0.10. WC applied to raw spectra was considered, showing an acceptable distance of 0.02, and practicable ranges for both substances. In the tentative release procedure the differentiation between Precirol® and Lubritab® based on raw spectra in the frequency range 10000–4000 cm^{-1} with a threshold of 0.98 was included. For the differentiation between the other substances, the tentative release procedure used WC applied to raw spectra in the frequency range 10000–4000 cm^{-1} with a threshold of 0.95 or WC applied to second derivative with a narrow frequency range of 7000–4000 cm^{-1} with a threshold of 0.95.

With all three WC-based methods, the paracetamol products of different particle size were not distinguished. Before starting with SIMCA, a PCA plot of all paracetamol samples was created to see if it was possible to distinguish the paracetamol samples based on particle size. The PCA

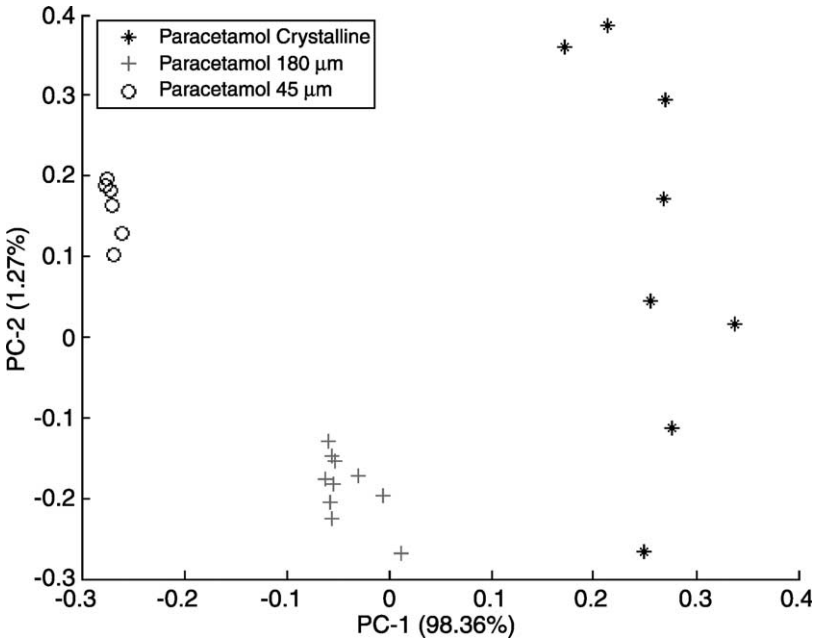

Fig. 2. PCA plot (PC1/PC2) of second derivative spectra (10000–4000 cm^{-1}) of paracetamol 45 μm, 180 μm and crystalline.

plot (PC1/PC2) showed three different clusters (see Fig. 2). PC1 describes the granular size in the paracetamol samples. Three SIMCA models were generated for each particle size and the models were validated for unequivocal identification with a probability level of 0.01. The calibration of these three models yielded a 100% recognition rate of each model and for the paracetamol substances, SIMCA with a probability level of 0.01 was included in the tentative release procedure after a pre-classification with WC. Testing first with WC is still required as the application of the method is not only to differentiate different grades of paracetamol but to verify the identity of the substances at issue, e.g. paracetamol from all relevant structure and name analogues.

The external validation I of the WC part of the tentative release procedures with independent batches of the 12 substances resulted in correlation coefficients larger than the thresholds of 0.98 or 0.95 (data not shown). No α-errors

Table 5
Results of the experimental external validation II of the tentative release procedure

Potential interfering substance	Raw spectra 10000–4000 cm^{-1}, threshold 0.98*		Second-derivative 7000–4000 cm^{-1}, threshold 0.95*		Conclusion[#]
	Correlation coefficient	Most likely substance	Correlation coefficient	Most likely substance	
Paracetamol 500-90	0.9943	Paracetamol	0.9982	Paracetamol	†
Hydrocortisone	0.5763	Cortisone acetate	0.2956	Prednisolone	OK
Dexamethasone	0.6300	Prednisone	0.2966	Macrogol 300	OK
Glycerol monostearate	0.9620	Precirol	0.9567	Precirol	OK
Acetylsalicylic acid	0.1078	Paracetamol	0.1670	Prednisolone	OK
Ethinyl estradiol	0.2890	Prednisone	0.2226	Furosemide	OK
Mesalazine	0.2284	Paracetamol	0.1769	Cortisone acetate	OK
Hydrocortisone acetate	0.6365	Prednisone	0.1653	Cortisone acetate	OK
Stearic acid pulverized	0.9281	Lubritab	0.8732	Precirol	OK
Prednisolone sodium phosphate	0.6169	Glycerol	0.3229	Prednisolone	OK
Ibuprofen	0.4779	Cortisone acetate	0.1789	Tolbutamide	OK
Testosterone propionate	0.6958	Cortisone acetate	0.2278	Cortisone acetate	OK
Clobetasone butyrate	0.7104	Prednisone	0.2269	Prednisone	OK
Triamcinolone	0.5208	Cortisone acetate	0.1611	Glycerol	OK
Betametasone dipropionate	0.7675	Prednisone	0.2338	Prednisone	OK
Beclometasone dipropionate	0.7461	Prednisone	0.2921	Prednisone	OK
Betametasone valerate	0.6297	Prednisone	0.1874	Prednisone	OK
4-Aminopyridine	0.1253	Cortisone acetate	0.1681	Furosemide	OK
Flumethasone pivalate	0.6526	Prednisone	0.2322	Precirol	OK
Prednisolone hemisuccinate	0.6619	Prednisone	0.2844	Prednisolone	OK
Glibenclamide	0.4768	Tolbutamide	0.1792	Paracetamols	OK
Sorbitol	0.4862	Glycerol	0.1998	Lubritab	OK
Cetylstearyl alcohol	0.7353	Lubritab	0.8187	Lubritab	OK
Fludrocortisone acetate	0.8381	Cortisone acetate	0.4733	Cortisone acetate	OK
Benzoic acid	0.3077	Paracetamol	0.1938	Cortisone acetate	OK
Progesterone	0.6818	Cortisone acetate	0.1543	Precirol	OK
Triamcinolone acetonide	0.5493	Prednisone	0.2192	Prednisone	OK
Salicylic acid	0.1812	Paracetamol	0.1536	Lubritab	OK
Polysorbate 80	0.8776	Macrogol 300	0.7122	Macrogol 300	OK
Propylene glycol	0.6726	Macrogol 300	0.2482	Precirol	OK
Glycerol	0.6465	Macrogol 300	0.8442	Glycerol	OK
Ethyl glycol	0.8475	Macrogol 300	0.4116	Macrogol 300	OK
Cetiol	0.8041	Precirol	0.4686	Precirol	OK
Paraffin liquid	0.8101	Precirol	0.5029	Precirol	OK
Macrogol 400	0.9934	Macrogol 300	0.9651	Macrogol 300	†
Betamethasone	0.7207	Cortisone acetate	0.3511	Cortisone acetate	OK
Methylprednisolone	0.5296	Prednisone	0.2140	Tolbutamide	OK
Gliclazide	0.4351	Prednisone	0.3059	Prednisone	OK
Prednisolone acetate	0.7501	Prednisone	0.2451	Prednisone	OK
Cortisone	0.8026	Cortisone acetate	0.4269	Prednisone	OK
Deoxycortone	0.7375	Cortisone acetate	0.1585	Lubritab	OK
4-Aminosalicylic acid	0.0709	Paracetamol	0.1246	Cortisone acetate	OK
Phenacetin	0.5605	Paracetamol	0.2653	Lubritab	OK
Tolazamide	0.5116	Tolbutamide	0.4014	Tolbutamide	OK
Bumetanide	0.3115	Furosemide	0.1999	Cortisone acetate	OK

* Threshold of the tentative release procedure; [#] OK = correctly rejected or accepted; [†] important result (see Section 3).

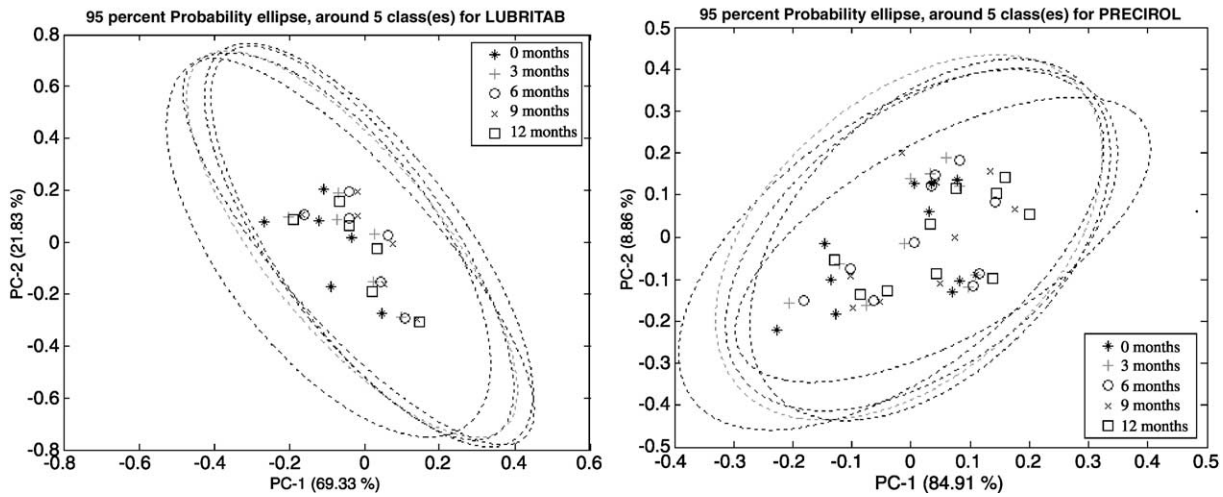

Fig. 3. PCA plots (PC1/PC2) and the related 95% confidence intervals of Precirol® and Lubritab® spectra recorded five times over a 1 year period.

or β-errors were obtained. The external validation I of the SIMCA part for the three paracetamol qualities resulted in correct identification of the independent validation batches with a probability level of 0.01.

The results of external validation II of the tentative release procedure are shown in Table 5. Glycerolmonostearate was correctly rejected using WC on raw data and a threshold of 0.98 being the tentative release procedure for Precirol® and Lubritab®. The similarity between the NIRS spectra is shown in Fig. 1.

The match of macrogol 400 with macrogol 300 showed an incorrect identification with both WC methods. It was concluded that the tentative release procedure, based on NIRS only, was not able to differentiate between the different homologues of macrogol. So, an additional test for the verification of the identity of macrogol 300 was included in the final release procedure. For the other substances both WC methods performed adequately. Paracetamol 500-90 was identified as paracetamol and by the subsequent test with SIMCA correctly rejected.

The robustness of the NIRS part of the procedure was tested with the substances Precirol® and Lubritab®, being the most critical pair of substances in the release procedure. The resulting PCA plots and 95% confidence intervals are shown in Fig. 3. The variance within the spectra of ten batches recorded at the same time was larger than the variance within the spectra of one batch recorded at five different times. Also, the 95% confidence intervals overlap and no differences are seen among the data collected during the 12 months. The results of the robustness testing of the WC part of the procedure both on raw spectra and on second derivative spectra are shown in Table 6. The results show that mean correlation is larger and the standard deviation and range are smaller using raw data in the spectral range

Table 6
Results of robustness testing of the WC part of the release procedure

Time (months)	Raw data 10000–4000 cm^{-1}			Second derivative 7000–4000 cm^{-1}		
	Mean correlation	Standard deviation	Range	Mean correlation	Standard deviation	Range
Precirol®*						
0	0.9995	0.0004	0.0014	0.9923	0.0057	0.0178
3	0.9996	0.0003	0.0006	0.9932	0.0029	0.0086
6	0.9995	0.0002	0.0006	0.9928	0.0022	0.0063
9	0.9996	0.0002	0.0006	0.9934	0.0027	0.0094
12	0.9996	0.0001	0.0003	0.9922	0.0028	0.0076
Lubritab®#						
0	0.9996	0.0003	0.0007	0.9955	0.0037	0.0090
3	0.9996	0.0002	0.0004	0.9955	0.0032	0.0082
6	0.9996	0.0002	0.0004	0.9967	0.0035	0.0035
9	0.9996	0.0003	0.0005	0.9953	0.0070	0.0070
12	0.9994	0.0003	0.0009	0.9923	0.0043	0.0107

* Eight samples were analyzed; # five samples were analyzed.

Table 7
Labelling, analytical result of the samples used in the practical challenge of the final release procedure and identity

Labelling	Result release procedure	Identity	Conclusion[#]
Precirol®*	Identified substance	Precirol®	OK
Lubritab®*	Identified substance	Lubritab®	OK
Furosemide	Identified substance	Furosemide	OK
Tolbutamide	Identified substance	Tolbutamide	OK
Prednisone	Detected mislabelling	Cortisone acetate	OK†
Prednisolone	Identified substance	Prednisolone	OK
Cortisone acetate	Identified substance	Cortisone acetate	OK
Paracetamol 180 μm*	Identified substance	Paracetamol 180 μm	OK
Paracetamol 45 μm	Identified substance	Paracetamol 45 μm	OK
Paracetamol crystalline	Identified substance	Paracetamol crystalline	OK
Glycerol 85%	Identified substance	Glycerol 85%	OK
Macrogol 300*	Identified substance	Macrogol 300	OK
No label	Identified substance	Paracetamol	OK
No label	Found no match	Hydrocortisone acetate	OK
No label	Found no match	Triamcinolone acetonide	OK
No label	Found no match	Paracetamol 500-90	OK†
No label	Identified substance	Precirol®	OK
No label	Identified substance	Prednisone	OK
No label	Identified substance	Paracetamol 180 μm	OK
No label	Identified substance	Lubritab®	OK
No label	Found no match	Miconazol	OK
No label	Identified substance	Tolbutamide	OK
No label	Found no match	Propylenglycol	OK
No label	Found no match	Sorbitol	OK

* Samples from batches that were also used for the calibration; † important result (see Section 3); # OK = correctly rejected or correctly accepted.

10000–4000 cm^{-1}. However, no differences were found on both methods among the five series of spectra recorded over a period of 1 year. So, the WC part of the procedure is robust in time. This is mainly due to the fact that the libraries were constructed over a longer period of time. We noticed a decrease of the mean correlations in time when using a library with spectra all recorded on 1 day [19].

The SIMCA part of the release procedure was not tested with paracetamol for which it was developed but also with Precirol® and Lubritab®, as we expected in this way to get more insight in the robustness over time in general for SIMCA-based differentiations. The technique was identical to the technique applied for the paracetamol differentiation in the final release procedure. We conclude that also the SIMCA part of the release procedure is robust in time when the models are carefully constructed with spectra recorded over a longer period of time.

The combined results of the external validation and the robustness tests demonstrate that the tentative release procedure is suitable. For WC applied on raw spectra the ranges are smaller, yet for WC applied on the second derivative the distances are larger. For convenience, i.e. no pre-treatment and one WC method for all substances, and to challenge a method different to the preferred method of the NfG, WC applied on raw spectra was chosen as the final release procedure for Precirol® and Lubritab® and for all other substances. This final release procedure was put into practice and the results are shown in Table 7. All samples were correctly identified, and no α-errors or β-errors were observed. In particular, this final release procedure detected one falsely labelled sample and rejected correctly a paracetamol particle size that was not included in the final release procedure.

4. Conclusions

In summary, we conclude that the dNfG has good practicability. The choices we made (as allowed by the NfG) and the developed method we investigated, WC on raw data and SIMCA as a second chemometric technique, did give good results.

We confirm the need for the requirement of the dNfG to carry out an external validation II: by this external validation II we detected an incorrect identification. Allowing such an external validation II by theoretical considerations in appropriate cases worked satisfactorily.

We also confirm the necessity of a two-step method. WC, with the calculation filter applied, focuses on spectral features and chemical properties and suppresses physical features. Therefore, the second chemometric technique (pre-treatment and algorithm) should cover differences due to the physical state of the substance. The dNfG recommends the use of MWD as a second chemometric technique, but other techniques may also provide good results as the validation showed.

We conclude that setting up a release procedure according to the dNfG is practical. However, a lot of work needs to be invested. For some industries an attractive option may be to verify the identity of one container of a supply by the EP tests, but assuring the correct identity of the other containers by a comparison of its NIR-spectra. For such a relatively simple application, there is no need to build a large reference library, while it still offers a major saving in time and an important gain in product security.

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