APPLICATION NOTE

Automation of the picoSpin 80 ^{1}H NMR benchtop spectrometer for high-throughput determination of the research octane number of fuels

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Abstract
The fully automated sample workflow, i.e. the preparation, injection, ^{1}H NMR spectral acquisition and sample cell purging, for determining the research octane number (RON) of fuels by means of low-field ^{1}H NMR spectroscopy (80 MHz proton Larmor frequency) is described. To this aim, a Thermo Scientific™ picoSpin™ 80 ^{1}H NMR benchtop spectrometer is coupled to a GERSTEL® MultiPurpose Sampler MPS (GERSTEL GmbH & Co. KG) in combination with the MAESTRO software. The automated sample preparation by the MPS involves the mixing of fuel samples and NMR reference compounds. After sample preparation and injection into the picoSpin flow cell via standard LC tubing, MAESTRO software transfers the sample ID as file name and initiates the NMR data acquisition. After termination of the NMR experiment, the tubing and cell are rinsed and prepared for the subsequent sample by the MPS.

The complete workflow is described for the classification and quantitative analysis of fuel samples by multivariate chemometric analysis, Principle Component Analysis (PCA) and Partial Least Squares analysis (PLS), to determine RON.

Introduction
Knock resistance of petrol is defined by the research octane number (RON).[1] Here, the knock indicates a spontaneous onset of explosion of the fuel-air mixture that is not triggered by the ignition spark, but by the high compression ratio.[2] This uncontrolled explosion can lead to damage of the engine components. A controlled fuel combustion usually occurs at a high knock resistance value for the petrol.

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To meet the high-quality requirements of fuels for modern combustion engine, product streams from refinery processes are analyzed before delivery with respect to the components contained.[3] The large number of samples requiring analysis demands an automated solution for sample preparation and analysis where the time factor plays a special role in the product release.[4] For motor protection, fuel additives are also added to prevent corrosion and to increase anti-knock properties.[5]

The current standard analysis for determining RON relies on the Cooperative Fuel Research (CFR) engine or gas chromatography, which are both usually slow methods for determining the RON for any liquefied petroleum gas mixture.[6-9] In this note, a fast NMR method is described to quickly derive RON by using the low-field benchtop picoSpin 80 ^{1}H NMR spectrometer where its NMR flow cell has been automated with a GERSTEL multipurpose sample (MPS) robot. The GERSTEL MAESTRO software controlled the MPS and initiated the NMR data acquisition through an executable. The samples were prepared and injected from 10 mL glass vials with septum closure arranged in a 32 position sample tray. The procedure was fully automated through this coupling. Subsequently, multivariate data analysis was applied. The analyzed fuels were classified according to their composition by means of principal component analysis (PCA). Quantitation was achieved by principal component regression, which was validated following the leave-one-out cross-validation method.[10-12]

Experimental

The picoSpin 80 ^{1}H NMR benchtop spectrometer was operated at 82 MHz proton Larmor frequency and at T = 34 °C. Its sample cell had a total volume of 40 μL and an active volume of 0.2 μL, which was suitable for flow experiments. Due to the effective sensitivity and an electronic lock system, neat liquids can be investigated without the need for deuterated solvents. The picoSpin 80 flow cell was connected via standard PEEK tubing to a GERSTEL MultiPurpose Sampler MPS 3, length 80 cm, equipped with a 1 mL syringe, a VT 32 rack for 10 mL vials, a Vici® Cheminert™ LC-valve and a Fast Wash Station (SpectraLab Scientific, Inc.) using methanol as the wash solvent. The MPS was controlled by GERSTEL MAESTRO software version 1.4.2.25. An example set-up of the MPS coupled to the picoSpin 80 spectrometer is shown in Figure 1.

Both devices were operated using a laptop computer running under Windows® 7 software. MAESTRO software controlled the MPS and initiated the NMR spectral acquisition via an executable, which was invoked automatically as a shell script from a command line in the MAESTRO program. All MPS methods and steps as well as the sample sequence, and the sample unique identifier were defined in MAESTRO software.

A sample volume comprising all the tubing and the sample cell volume (i.e. in our set-up a little less than 1 mL) was drawn from 10 mL sample vials in the 32-tray of the MPS by the 1000 μL-glass syringe and inserted into the injection valve. The liquid sample was then driven to the flow cell through a PEEK capillary (Figure 1).

Figure 1: Low-field picoSpin 80 ^{1}H NMR spectrometer with a MultiPurpose Sampler (GERSTEL) for automated sample preparation and injection of fuel samples for determining the research octane number (RON).

The NMR experiment was started as soon as MAESTRO software switched the multi-port valve. The start signal was passed to the NMR spectrometer via a LAN cable. The NMR acquisition program "one-pulse," which was initiated from the executable within the MAESTRO software and started the NMR experiment, is part of the picoSpin 0.9.1 software version.

The fuels were then transferred into the NMR spectrometer via the multi-port valve of the MPS with 100 μL of tetramethylsilane (TMS) added as reference without prior dilution by deuterated solvents. As stated above, a turn of this valve was interpreted as the start command by the executable, and this initiated the NMR experiment that was defined in the picoSpin software.

For this study, a simple one-pulse experiment followed by acquisition and a sufficiently long relaxation delay, i.e. 7 s, were employed. Sixty-four spectra per sample were accumulated. The NMR program stored the resulting digitized free induction decays (FID) in a file of 4 K data points each and copied them into a predefined directory on the laptop computer where MestReNova® 9.0.1 software (Mestrelab Research Inc.) processing was performed on the same computer. The sample was removed and transferred to waste through injection of the following sample and overfilling of sample cell and tubing. The overall experiment time was about 7 min per sample. The standard RON determination using the combustion motor usually takes about 25 min per sample without warming-up period, with the possible reduction of the latter if a sample series is tested. Common gas chromatographic (GC) methods employ run times between 45 up to 90 min.

Using this MPS and picoSpin 80 configuration, a total of 81 fuel samples were investigated. The spectra were prepared for PCA and PLS and analyzed using MATLAB® version R2015b (MathWorks, Inc.).

The combined automation of sample preparation, injection and analysis would allow the system to process more than 200 samples in a 24 h period. For a truly automated workflow without any requirement for manual interference, the MPS would need to be equipped with multiple sample trays. The sequence of processing and spectral recording of the fuel samples was programmed graphically through MAESTRO software. The function "prep ahead" allowed the following sample to be prepared while NMR experiments of the current sample were recorded. For a complete sample tray, a total of 90 minutes can thus be economized.
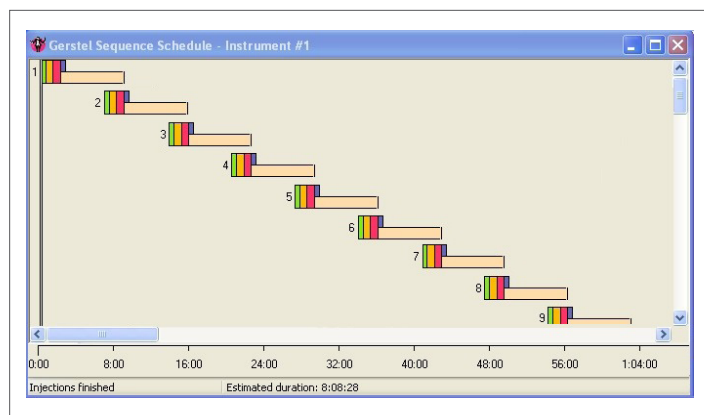
Figure 2: Graphical representation of the automated RON determination workflow in the GERSTEL MAESTRO Scheduler program. After adding TMS as a reference substance (green), the sample is mixed (orange), and then injected (red). The syringe is rinsed (purple) during the measurement (apricot).

A graphical representation of the automatically staggered "Prep-and-Shoot" workflow within MAESTRO Scheduler program is displayed in Figure 2.
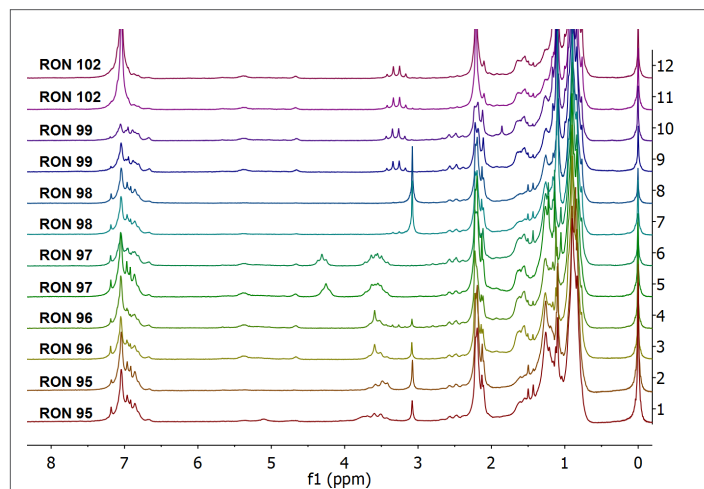
Figure 3: Low-field ¹H NMR-spectra (80 MHz, T = 34 °C) of neat fuels with different RONs.

Results and discussion

A collection of the ¹H NMR spectra of twelve fuel samples with research octane numbers between 95 and 102 is shown in Figure 3. RONs are rounded scientifically to the nearest integer.

At first glance the spectra appear quite similar. Aromatic signals near 7 ppm from the aromatic protons resonances of benzene, toluene, and xylene, all common components of fuel are clearly visible and difficult to distinguish, as are the aliphatic resonances of the CH_3, CH_2 and CH groups found between 0.6 ppm and 2.3 ppm (Figure 3). However, closer examination reveals a number of resonances characteristic of the predominant additives used to adjust RONs and comply with national regulations on commercial fuels.

The predominant additives, that are typically used to adjust RONs and comply with national regulations in commercial fuels, are ethanol, methyl-*tert*-butylether (MTBE), and ethyl-*tert*-butylether (ETBE). In the spectra of fuels having RON 95 and RON 98 a strong signal arising from methyl-*tert*-butylether (MTBE) was observed at 3.1 ppm. Fuel spectra of RON 99 and RON 102 exhibited a quartet signal around 3.3 ppm stemming from ethyl-*tert*-butylether (ETBE). In lower RON fuels, ethanol signals at 4.2 and 1.2 ppm were regularly observed.

When inspecting the stacked spectra in Figure 3, it is obvious that an easy classification of the various fuel samples with respect to RON is not possible. Therefore, a principal component analysis (PCA), as a well-known and

hence widespread, simple yet robust method, was chosen from the variety of multivariate data analysis techniques to distinguish fuels according to their RON (Figure 4).
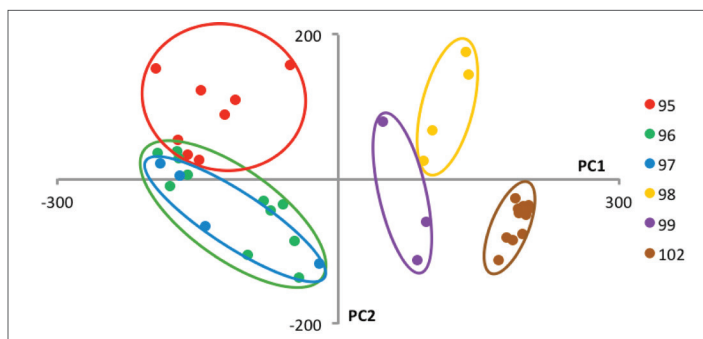
Figure 4: Results of PCA using the ^{1}H NMR spectra of 47 different fuel samples with RON 95 (red), 96 (green), 97 (blue), 98 (yellow), 99 (purple) and 102 (brown).

Using PCA, four RON ranges were clearly distinguishable. A very good separation was achieved for RON 102. To a slightly lesser extent, RON 98 and 99 fuels were found to be separate as well. The spectra with lower RON had features too similar, i.e. ethanol content varying from 4-9% volume. Hence, samples with RON 95, 96, and 97 could not clearly be separated from each other.

A multivariate Partial Least Squares regression (PLS or PLS-R) goes beyond univariate calibration models and is therefore appropriate for the quantitative analysis starting from complex spectra. Multicomponent systems with varying concentrations of different components can be examined. For the samples under investigation, a suitable model could be established and tested using the data set containing 27 samples. Using a leave-one-out validation approach, the RON of the ith sample, i.e. the left-out one, was predicted. The results yielded RON values with a deviation of 0.2 or smaller for approximately 31% of the samples, a deviation between 0.2 and 0.5 for just over 48%, between 0.6 and 0.7 another 14% and a deviation over 0.8 for the remaining 7%. The 27 predicted RONs vs. those obtained by the combustion engine are shown in Figure 5. A chart of the deviations is displayed in Figure 6 for the individual values. This model yielded rather accurate quantitative information, whereas the PCA allowed for qualitative discrimination. Hence, PLS was considered a promising starting point for further studies applying more sophisticated chemometric methods.

Conclusions

The MPS and MAESTRO system enabled the picoSpin 80 NMR spectrometer to be automated for batch analyses. The MPS of 80 cm length fits the picoSpin 80 spectrometer in size, whereas a larger MPS provides

further options for a wider range of applications (e.g. more sample trays, a switching valve to add a GC or GC-MS). The system can be fully controlled via MAESTRO software including an executable for communicating with the picoSpin software. Only the processing and interpretation of the NMR spectral data requires further software, such as MestReNova, from other vendors. The MPS-picoSpin 80 combination was used to record ^{1}H NMR spectra of 47 fuel samples and was shown to be a fast, high-through-put method for calculating RONs. Qualitative multivariate data analysis was applied on the spectra to classify the samples according to their RON. The distinction of fuels according to their RON proved successful especially for RON 98 to 102. Quantitative PLS yielded a fairly accurate prediction of RONs considering the simplicity of the approach and the ease of using the MPS, MAESTRO and picoSpin NMR combination.
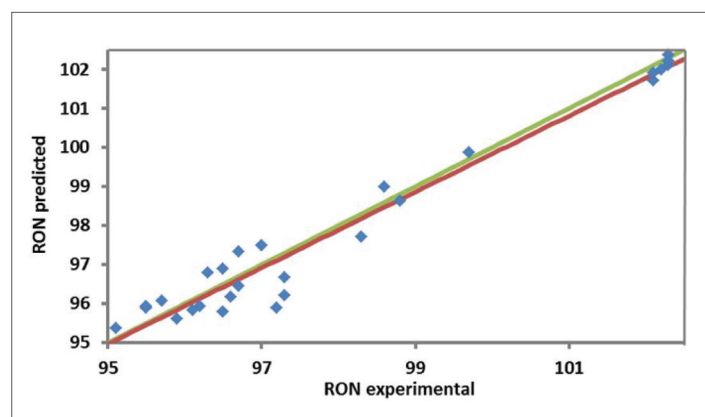
Figure 5: Predicted RON from the ^{1}H NMR spectra of 27 samples vs. combustion derived RON using a PLS model based on a calibration set of 47 samples; theoretical bisector (green) and experimental linear function y = 0.97x+2.49.
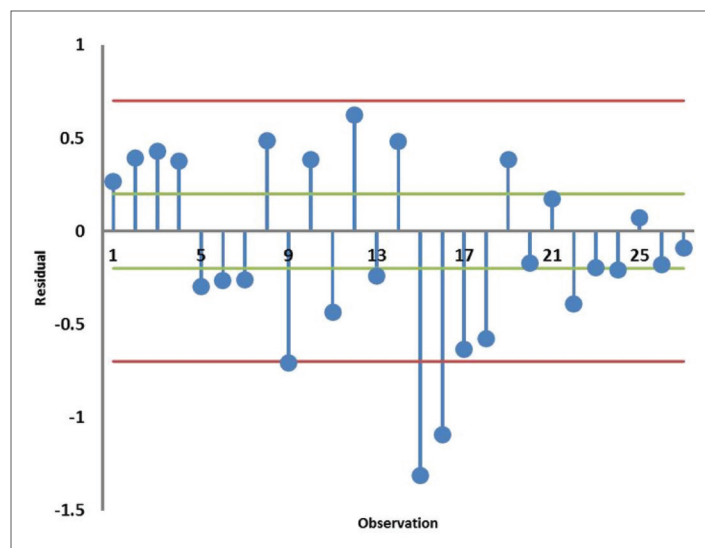
Figure 6: Residual plot obtained from the PLS of the 27 validation samples. Deviations are shown according to ASTM D2885-13 as follows: Repeatability ≤ 0.2 (green), reproducibility ≤ 0.7 (red).

MPS:	
Syringe:	1000 µL
Injection volume:	700 µL
Injection speed:	50 µL/s
Pre wash:	2
Mixing:	1
Post wash:	2
Injection delay:	8 min

Table 1: Set-up parameters of the sample treatment within the GERSTEL MAESTRO Scheduler as used for the RON determination of fuel samples.

Low-field ^{1}H NMR	
Frequency:	82 MHz
Number of accumulations (Scans):	64
Pulse length:	58 µs
Acquisition points:	4096
Recovery delay:	500 µs
Recycle delay:	7 s
Bandwith:	4 kHz
Post Filter Attenuation:	11
Zero filling:	9000

Table 2: Experimental parameters for the ^{1}H NMR experiment with the picoSpin 80 ^{1}H NMR spectrometer as used for the RON determination of fuel samples.

References

[1] DIN EN 228 - Kraftstoffe für KFZ - Unverbleite Ottokraftstoffe- Anforderungen und Prüfverfahren, 2014.

[2] D. Gruden, Umweltschutz in der Automobilindustrie: Motor, Kraftstoffe, Recycling, 45-250 Teubner-Verlag, Wiesbaden, 2008.

[3] DIN 51756 - Prüfung von Ottokraftstoffen- Bestimmung der Klopffestigkeit (Octanzahl) - Allgemeines, 1990.

[4] W. Chew, P. Sharratt, Trends in process analytical technology, Anal. Methods. 2 (2010) 1412–1438.

[5] W. Eifler, E. Schlücker, U. Spicher, G. Will, Küttner Kolbenmaschinen, 7th ed., 257-512 Teubner-Verlag, Wiesbaden, 2009.

[6] ASTM D2885-13 - Standard Test Method for Determination of Octane Number of Spark-Ignition Engine Fuels by On-Line Direct Comparison Technique , 2013.

[7] P. Mello, F. Wildner, G.S. de Andrade, R. Cataluna, R. da Silva, Combustion time of the oxygenated and non-oxygenated fuels in an Otto cycle engine, J. Brazilian Soc. Mech. Sci. Eng. 36 (2014) 403–410.

[8] T.G. Leone, J.E. Anderson, R.S. Davis, A. Iqbal, R.A. Reese, M.H. Shelby, et al., The Effect of Compression Ratio, Fuel Octane Rating, and Ethanol Content on Spark-Ignition Engine Efficiency, Environ. Sci. Technol. 49 (2015) 10778–10789.

[9] V.H. Rapp, H. Mack, P. Tschann, W. Hable, R.J. Cattolica, R.W. Dibble, et al., Research octane numbers of primary and mixed alcohols from biomass-based syngas, Energy and Fuels. 28 (2014) 3185–3191.

[10] K.E. Nielsen, J. Dittmer, A. Malmendal, N.C. Nielsen, Quantitative Analysis of Constituents in Heavy Fuel Oil by ^{1}H Nuclear Magnetic Resonance (NMR) Spectroscopy and Multivariate Data Analysis, Energy & Fuels. 22 (2008) 4070–4076.

[11] R.M.R. Tainara, L.F. Danilo, B. Nivaldo, E.D.O. José, ^{1}H NMR fingerprinting of brazilian commercial gasoline: Pattern-recognition analyses for origin authentication purposes, Energy and Fuels. 23 (2009) 3954–3959.

[12] S. Wold, M. Sjöström, L. Eriksson, PLS-regression: A basic tool of chemometrics, Chemom. Intell. Lab. Syst. 58 (2001) 109–130.

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