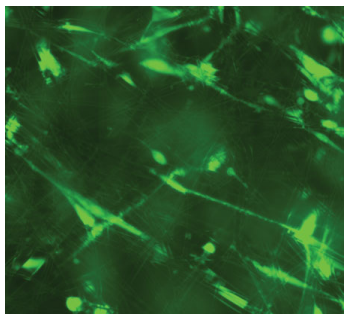
KNAUER

# Application Note



# Determination of biocompatible Polymer building Monomers

| | |
|---|---|
| **Category** | Biosciences |
| **Matrix** | - |
| **Method** | UHPLC |
| **Keywords** | Biopolymer, Biomaterial, Implants, Monomer, Hydrogel |
| **Analytes** | Ethylenglycol dimethacrylate (EGDMA), 2-Hydroxyethyl methacrylate (HEMA), Methacryloxymethyltrimethylsilane (TMS-MA), Methyl methacrylate (MMA), N-Vinyl-2-pyrrolidon (N-VP), Styrene |
| **ID** | **VBS0021N**, 10/2012 |

PLATIN blue


## Summary

A fast gradient method for the separation and quantification of six substances used as monomers for the production and development of biocompatible polymers is presented in this application note. The high speed and reliability of this method applying the KNAUER PLATINblue UHPLC System make it well-suited as well for routine analyses as for research tasks. The very short analysis time of less than 7 minutes is achieved by employing the Bluespher phenyl stationary phase with a 2 µm particle size filled in a 2 mm ID column. A binary high pressure gradient instrumentation is used at a flow rate of 0.3 ml/min in combination with the PDA-1 UV-detector.


## Introduction

In medical sciences, a wide field for the use of biomaterials exists. A biomaterial is defined as any synthetic material that is used to replace or restore function to a body tissue and is continuously or intermittently in contact with body fluids.[1] This definition is somewhat restrictive, because materials used for devices like surgical or dental instruments have also to be regarded.[2] Independent of the way of use, biomaterials and especially implants have to fulfil some requirements that can be summed up as biocompatibility, mechanical stability and manufacturing properties. For this reason, there is a lot of research going on in the field of creating new biomaterials today.

In general, Metals and alloys, Ceramics and glasses, Polymers and Composites are used as biomaterials. Today, research often focuses on the development of biocompatible polymers, caused by their wide application area ranging from facial prostheses to tracheal tubes, from kidney and liver parts to heart components, and from dentures to hip and knee joints. Polymeric materials are also used for medical adhesives and sealants and for coatings that serve a variety of functions.[2] A special polymer class is depicted by hydrogels that are in many cases used in ophthalmics. Here, a natural lens destroyed by cataracts for example can be replaced by a synthetic polymer lens.[3]

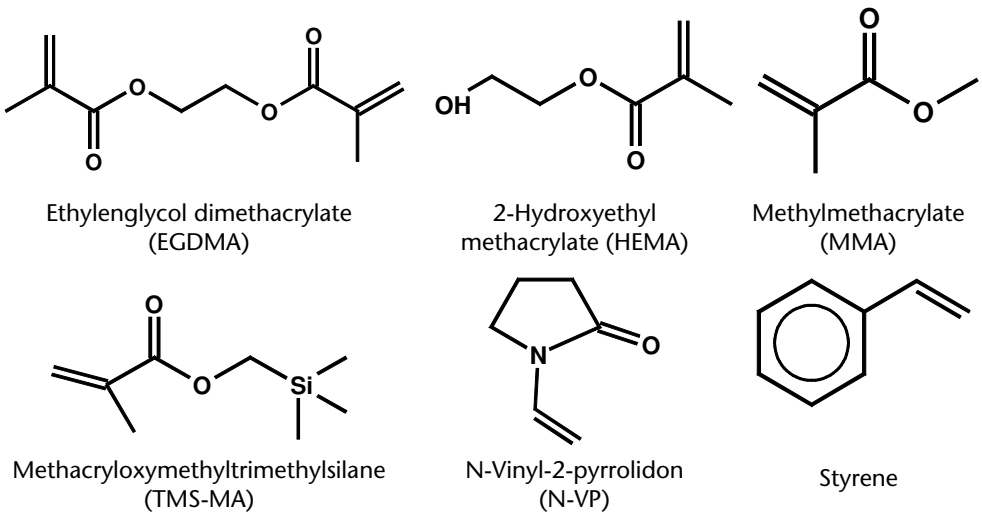
In this application note, a UHPLC method for the easy and fast determination of the composition of a range of substances used in research tasks for the production of innovative biopolymers that can be used as biocompatible implants is described. The very short acquisition time and reliability of the method show its good adequacy for routine analyses as well as for ambitious research challenges.

## Experimental preparation of standard solution

Single substance stock solutions for the identification of every peak were assembled by dissolving a small amount of every compound in acetonitrile.
A mixed stock solution was prepared by weighing out every substance exactly. Acetonitrile was used as the first dissolving solvent and concentrations in the stock solution were realized according to the following table.

| Substance | Concentration in stock solution [mg/ml] |
|---|---|
| EGDMA | 4.18 |
| HEMA | 3.78 |
| MMA | 4.44 |
| N-VP | 3.38 |
| Styrene | 4.80 |
| TMS-MA | 4.02 |

All standard solutions were prepared by diluting the stock solution using acetonitrile as the diluting solvent. Dilutions were 1:100, 1:500, 1:1000 and 1:2000, so that concentrations were realized for calibration in the range of about 1 – 50 µg/ml.




Ethylenglycol dimethacrylate (EGDMA)

2-Hydroxyethyl methacrylate (HEMA)

Methylmethacrylate (MMA)

Methacryloxymethyltrimethylsilane (TMS-MA)

N-Vinyl-2-pyrrolidon (N-VP)

Styrene

**Fig. 1**

Chemical structures of the analyzed substances

## Method parameters

| | | | |
|---|---|---|---|
| **Column** | Bluespher 100-2 Phenyl, 100 x 2 mm | | |
| **Eluent A** | Water | | |
| **Eluent B** | Acetonitrile | | |
| **Gradient** | **Time [min]** | **% A** | **% B** |
| | 0.00 | 80 | 20 |
| | 3.00 | 10 | 90 |
| | 5.00 | 10 | 90 |
| **Flow rate** | 0.3 ml/min | | |
| **Injection volume** | 1 µl | | |
| **Column temperature** | 40 °C | | |
| **System pressure** | approx. 500 bar | | |
| **Detection** | UV at 215 nm (50 Hz, 0.02 s) | | |
| **Run time** | 7 min | | |

## Results

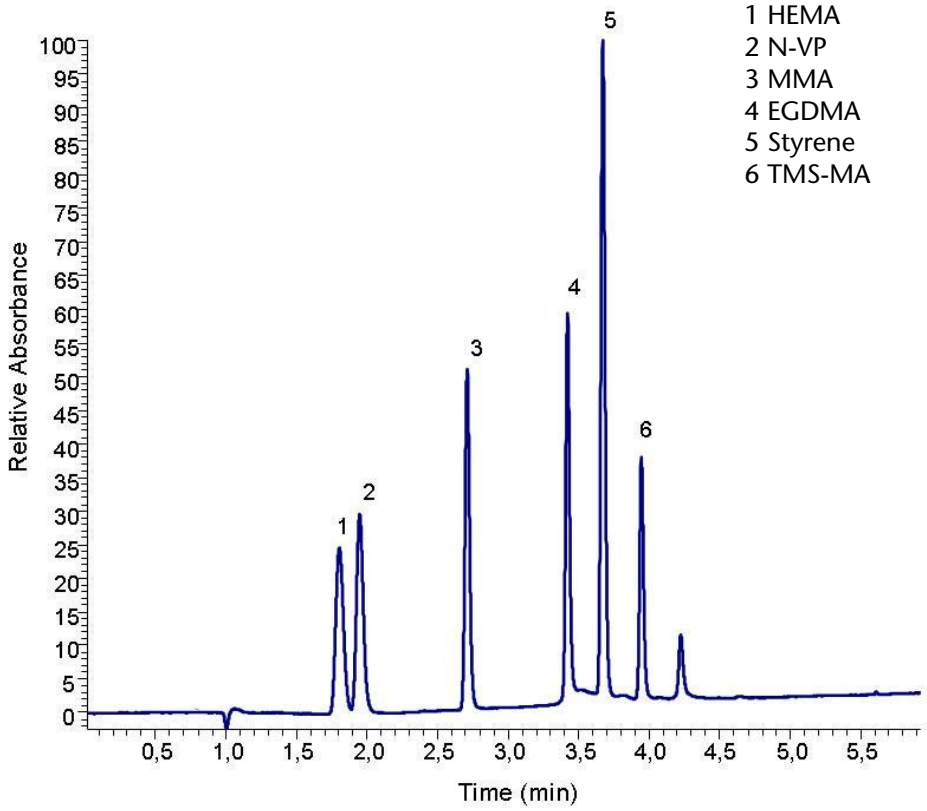



**Fig. 2**

Separation of the standard mixture containing 6 monomers. Chromatogram was generated using the Xcalibur software*

Figure 2 shows, that all substances could be baseline separated using the presented method with a Bluespher phenyl column. During method development, several stationary phases were tested, but not one of the applied polar endcapped C18A or C8AH phases was able to separate HEMA and N-VP sufficiently. The very special selectivity of the Bluespher phenyl phase was successful in the end.
It is apparent that an additional peak occurs after MMA. This is caused by an inhibitor that has to be added to the standard substances to abort polymerization. In general, the analyzed compounds are highly reactive to polymerization and have therefore to be stored in a dark and cold place with an inhibitor added to every filling.

As a second step, the method developed in this work was tested with regard to its reproducibility and stability. The peak areas and the retention times of nine replicate runs were statistically evaluated and the standard deviations were determined. The overlay of the nine corresponding chromatograms is shown in figure 3 and the statistical evaluation is shown in table 1.

After the stability and reproducibility of the method were proven, calibration was done with the prepared mixed standards.

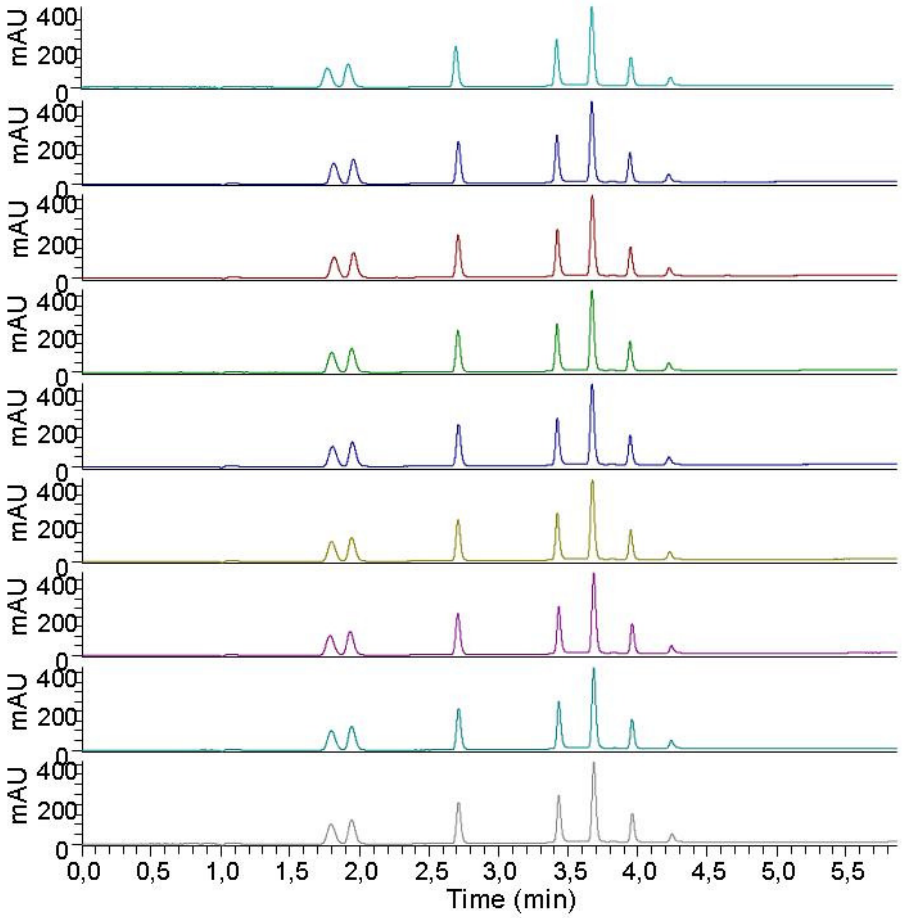



**Fig. 3**

Overlay of nine replicate chromatograms of the analysis of the standard mixture. Chromatograms were generated using the Xcalibur software*

**Table 1**

Statistical evaluation of the method´s reproducibility*

| Analysis | HEMA | | N-VP | | MMA | | EGDMA | | Styrene | | TMS-MA | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Nr. | Area | RT | Area | RT | Area | RT | Area | RT | Area | RT | Area | RT |
| 1 | 392 | 1,78 | 418 | 1,93 | 492 | 2,71 | 480 | 3,43 | 841 | 3,69 | 290 | 3,97 |
| 2 | 395 | 1,81 | 427 | 1,95 | 500 | 2,71 | 471 | 3,42 | 837 | 3,67 | 293 | 3,94 |
| 3 | 383 | 1,82 | 406 | 1,96 | 491 | 2,71 | 464 | 3,42 | 824 | 3,67 | 282 | 3,95 |
| 4 | 395 | 1,80 | 427 | 1,94 | 493 | 2,71 | 474 | 3,42 | 839 | 3,67 | 300 | 3,94 |
| 5 | 395 | 1,81 | 428 | 1,95 | 499 | 2,71 | 484 | 3,42 | 853 | 3,67 | 306 | 3,95 |
| 6 | 405 | 1,80 | 423 | 1,94 | 507 | 2,71 | 474 | 3,42 | 850 | 3,67 | 300 | 3,95 |
| 7 | 396 | 1,79 | 422 | 1,93 | 492 | 2,71 | 481 | 3,43 | 852 | 3,68 | 299 | 3,96 |
| 8 | 398 | 1,80 | 408 | 1,94 | 489 | 2,71 | 470 | 3,43 | 825 | 3,68 | 297 | 3,96 |
| 9 | 392 | 1,79 | 412 | 1,94 | 479 | 2,71 | 463 | 3,43 | 811 | 3,68 | 280 | 3,96 |
| Average | 395 | 1,80 | 419 | 1,94 | 493 | 2,71 | 473 | 3,43 | 837 | 3,68 | 294 | 3,95 |
| Std. Dev. [%] | 1,5 | 0,7 | 2,1 | 0,5 | 1,6 | 0,1 | 1,5 | 0,2 | 1,7 | 0,2 | 3,0 | 0,2 |

The relative standard deviation lies in the range of less than 1 % regarding the retention time and in the range of less than 3 % regarding the peak areas. These results underline the stability and robustness of the presented method.

Calibration was done for all six substances in the range of 1 – 50 µg/ml using the method of external standards. For every concentration, three replicate analyses were performed and the resulting calibration curves are shown in figure 4 - 9. The linear function and the correlation coefficient $R^2$ are shown above the curves. $R^2$ lies for all substances in the range of > 0.998.

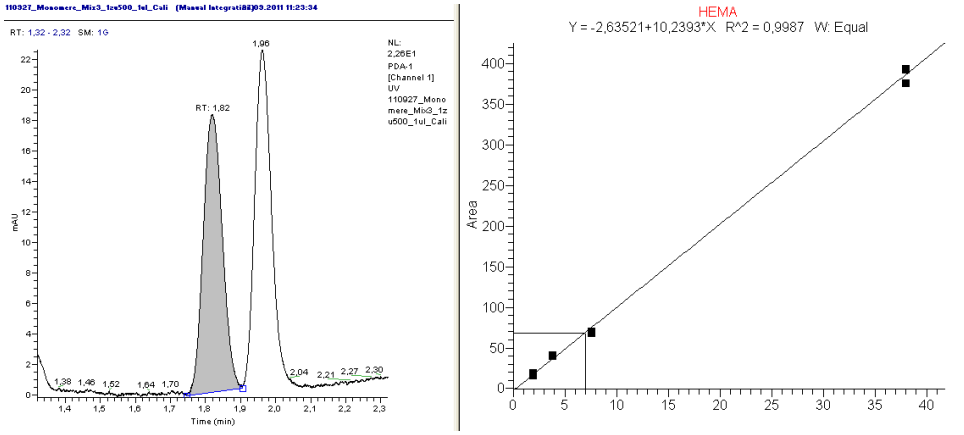



**Fig. 4**
Calibration curve* for HEMA

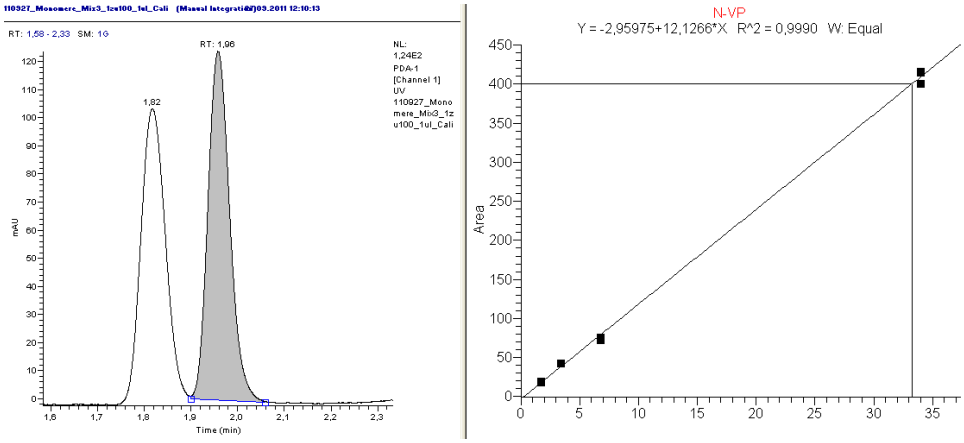



**Fig. 5**
Calibration curve* for N-VP

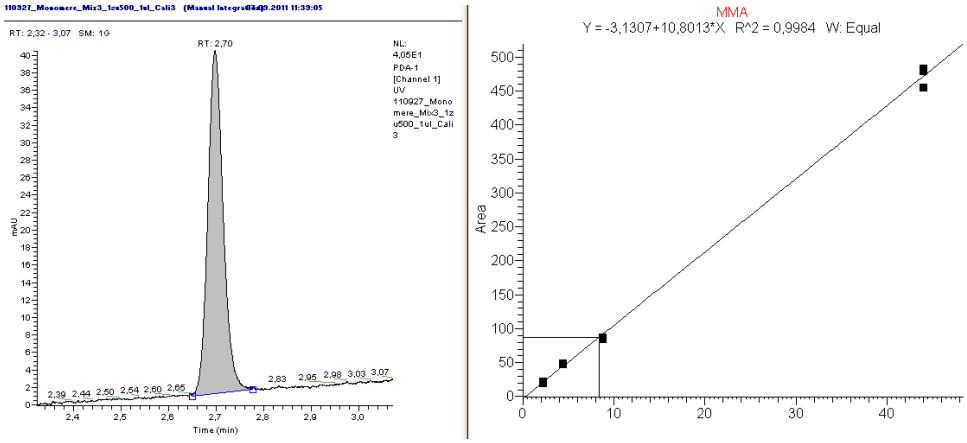



**Fig. 6**
Calibration curve* for MMA

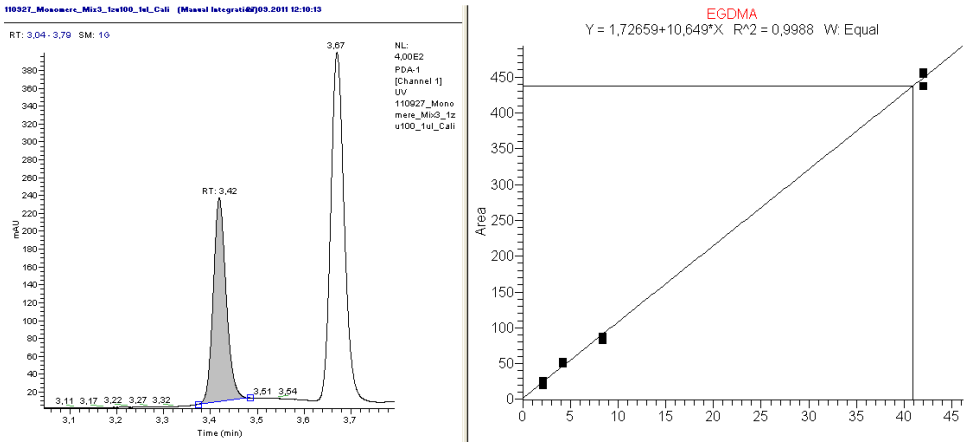



**Fig. 7**
Calibration curve* for EGDMA

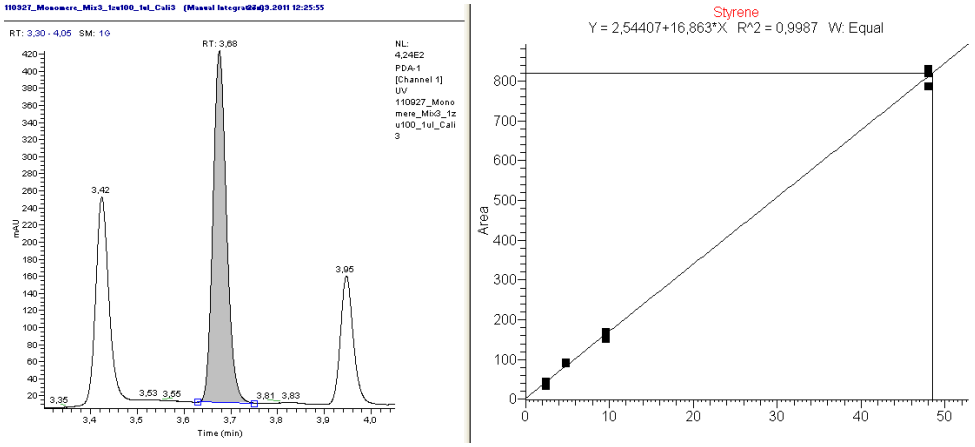



**Fig. 8**
Calibration curve* for Styrene

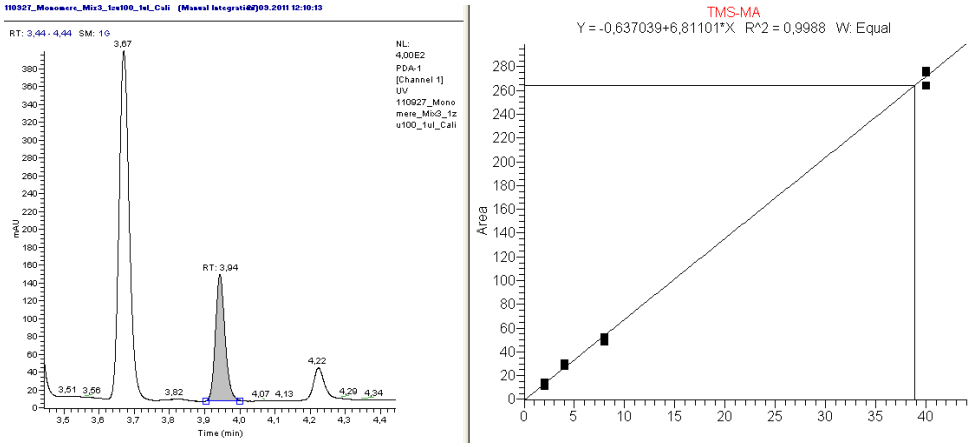



**Fig. 9**
Calibration curve* for TMS-MA

The limit of detection (LOD) lies for a signal to noise ratio of 3 (S/N=3) in the range of 0.18 – 0.57 µg/ml and the limit of quantification lies in the range of 0.54 – 1.71 µg/ml for a signal to noise ratio of 9 (S/N=9). Table 2 shows the values for every single compound.

**Table 2**
Detection- and Quantification limits

| Substance | LOD [µg/mL] (S/N = 3) | LOQ [µg/mL] (S/N = 9) |
|---|---|---|
| HEMA | 0.57 | 1.71 |
| N-VP | 0.43 | 1.28 |
| MMA | 0.35 | 1.04 |
| EGDMA | 0.29 | 0.86 |
| Styrol | 0.18 | 0.54 |
| TMS-MA | 0.43 | 1.29 |

*All chromatograms and calibration curves as well as all statistical data in this work were generated applying the Xcalibur software for PLATINblue. This software is not required for the application presented here, the same measurements can also be figured out with the KNAUER Chromgate Software package.

## Method performance

| | |
|---|---|
| **Limit of detection** | < 0.57 µg/ml range (S/N = 3) |
| **Linearity ($r^2$)** | > 0.998 |
| **Retention time precision*** | < 1 % RSD |
| **Peak area precision*** | < 3 % RSD |

*repeatability calculated over 9 replicate runs

## Conclusion

As a conclusion, the presented UHPLC method allows for the separation and quantification of six monomers in less than four minutes. Including a washing step and reequilibration of the column, only seven minutes are needed per run. That underlines that the method is well suited for routine analyses as well as for research tasks. Caused by the low eluent flow that is typical for UHPLC methods, only 2.1 ml eluent are needed for one analysis. This underlines the environmental compatibility and the economy of the presented method.

## References

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2. Joseph R. Davis, ASM International, Handbook of Materials for Medical Devices, Chapter 1: Overview of Biomaterials and Their Use in Medical Devices, Ohio, USA, 2003

3. Fraunhofer Institute for Applied Polymer Research IAP, Annual Report 2005/2006, http://www.iap.fraunhofer.de/institut/jbs/jb2005-2006.pdf

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## Physical properties of recommended column

Bluespher® columns are packed with ultra pure silica stationary phase to provide excellent separation performance and are well-suited for either routine analysis or ambitious chromatography in high speed mode where resolution, sensitivity and sample throughput are critical. These columns are your first choice for high-throughput-screening, quality control, and method development.

Bluespher Phenyl offers alternative selectivity for aromatic and moderately polar analytes or mixtures with varying polarity and aromaticity using π-π interactions with aromatics as the separation mechanism.

| | |
|---|---|
| **Stationary phase** | Bluespher 100-2 Phenyl |
| **USP code** | L 11 |
| **Pore size** | 100 Å |
| **Pore volume** | 0.8 ml |
| **Specific surface area** | 320 m²/g |
| **Particle size** | 2 µm |
| **Form** | spherical |
| **% C** | 12 % |
| **Endcapping** | yes |
| **Dimensions** | 100 x 2 mm |
| **Order number** | 10BE050BSF |

## Recommended Instrumentation

The high speed analysis was performed on the KNAUER high pressure gradient PLATINblue System, equipped with two pumps P-1, Degasser Unit M-1, Autosampler AS-1, Column Temperature Manager T-1 and Detector PDA-1.

| Description | Order No. |
|---|---|
| PLATINblue UHPLC-System | A69420 |
| PLATINblue Pump P-1 | |
| PLATINblue Pump P-1 with Degasser | |
| PLATINblue Autosampler AS-1 | |
| PLATINblue Column Thermostat T-1 Basic | |
| PLATINblue Detector PDA-1 | |
| PDA-1 flow cell (10 mm, 2 µl) | |
| PLATINblue CG Data system | |
| PLATINblue CG spectra license | |
| PLATINblue UHPLC method converter | |
| PLATINblue stainless steel capillary kit | |
| Optional: | |
| Xcalibur 2.1 Software | A66549 |
| PLATINblue driver for Xcalibur | A66801 |

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