An HPLC-DAD Method for Rapid and High Resolution Analysis of Concentrated BTEX and Styrene Aqueous Samples

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Abstract

A rapid HPLC-DAD method for analysis of concentrated BTEX and styrene (BTEXS) aqueous mixture is reported. Good resolutions of close to or greater than 1.5 were obtained for high equimolar BTEXS concentrations of up to 2.0 mM. At 5.5 min per sample analysis, this method is also one of the fastest HPLC methods to date, providing high throughput analysis and lowering analysis price.

Keywords: BTEX, Styrene, HPLC-DAD, Pluronic F-68

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Toxic and carcinogenic monoaromatic compounds such as benzene, toluene, ethylbenzene, xylenes (BTEX) and styrene, collectively termed "BTEXS", are present at high concentrations in many industrial effluents.[1-3] Improper treatment or disposal of these compounds will result in environmental pollution and bring about serious health and ecological repercussions.[4-6] Therefore, periodic environmental monitoring and remediation of contaminated waters is pivotal to environment and public health protection. To facilitate the work of field engineers, it is imperative to have an analytical method which is suitable for the analysis of concentrated aqueous BTEXS mixture.

Currently, gas chromatography (GC) is widely employed for analysis of monoaromatic-contaminated aqueous samples.[4] However, GC has limitations in its application to water samples with high BTEXS concentrations. This is due to its incompatibility with water, the potential detector saturation and compromised detection accuracies beyond 2.0 to 9.5 μM range.[7-8] Hence, GC-based methods are less well-suited for effluent monitoring or bioremediation studies where high BTEXS concentrations between 0.5 to 2.0 mM are often encountered.[5, 9-10] To circumvent these shortcomings, sample preparation and dilution or multi-step temperature ramp oven programme[4, 11] are often a necessity. These measures require costly accessories and can be time-consuming, lowering the analysis throughput. Furthermore, the volatile nature of BTEXS constitutes to sample loss during pre-analysis procedures and compromises measurement accuracy.

The limitations of GC based methods might be overcome by high performance liquid chromatography (HPLC). Unlike GC, HPLC is well-suited for the direct analysis of aqueous-

based samples and is able to accept higher compound concentrations. This eliminates the need for complex pre-analysis treatments and offers several advantages in terms of minimal sample loss, time-saving, higher throughput, and lower capital cost. Thus, HPLC is emerging as a popular tool to complement GC for BTEX analysis in water samples.[12-15] Methods based on reversed-phase C8 and C18 columns are commonly reported.[12, 14-15] Recently, a β-cyclodextrin stationary phase column was reported for BTEX analysis as well and has an advantage over C8/C18 columns in its ability to achieve complete separation of *m*-xylene and *p*-xylene.[13] However, the upper detection limit of these existing HPLC methods remains inadequate for analysis of high analytes concentrations.[12-13] This is due to the deterioration of resolution, especially between ethylbenzene and xylenes at high concentrations. Apart from BTEX, styrene is also known to be a prominent contaminant in petrochemical industrial wastewater.[5, 16] To our knowledge, while HPLC detection of styrene has been described[17], there is no HPLC method for the concurrent detection of styrene and BTEX, particularly when these compounds co-occur at high concentrations. To apply HPLC for the analysis of concentrated BTEXS aqueous samples, efforts to develop new methods are warranted.

In this communication, we report a fast and simple HPLC-DAD method suited for the analysis of aqueous samples containing high concentrations of BTEXS. The method is based on a newly-developed Acclaim Phenyl-1 reversed-phase column (4.6 x 150 mm, 3 µm) from Thermo Scientific Dionex (USA). The column stationary phase comprised of silanes bearing C11 alkyl aromatic moiety with a terminal electron-withdrawing group. The retention mechanism is based on a combination of hydrophobic and π-π interactions.[18] Operation parameters including mobile phase, sample injection volume and flow rate were determined and

optimized for rapid detection with high chromatographic resolution. Analytical parameters including linearity, limits of detection (LOD), limits of quantification (LOQ), repeatability and reproducibility were also investigated. The method was also applied for BTEXS analysis in an actual wastewater sample and recovery analysis of high BTEXS concentration in several types of aqueous matrices.

We first tested various solvent mobile phases for the chromatographic separation of BTEXS mixture at a high equimolar concentration of 2.0 mM. The solvent mobile phases chosen for testing were methanol, ethanol and acetonitrile, which are solvents commonly used in the HPLC detection of aromatic compounds.[12, 19-21] The solvent/water (%/%) compositions were varied between 50/50 and 70/30 at isocratic mode. Standard solution of BTEXS mixture was prepared by mixing the compounds together at equimolar concentrations before diluting in methanol to the desired concentrations and kept chilled at 15 °C. HPLC analysis was performed on UltiMate 3000 HPLC equipped with a DAD detector (Thermo Scientific Dionex, USA). The detection wavelength was set at 201 nm where the compounds showed peak UV absorbance during a wavelength scan from 190 nm to 300 nm (data not shown). The column oven temperature was programmed at 50 °C according to the manufacturer's recommendation. The best separation was achieved using methanol as the mobile phase with six resolved peaks ascribed, in sequence of elution, to benzene, toluene, styrene, ethylbenzene, *o*-xylene, and *m*-, *p*-xylene (Fig. 1).

To determine the range of methanol content that produces fast separation with reasonable chromatographic resolution, methanol/water (%/%) compositions between 50/50 and 70/30 were studied. Increasing methanol content from 50 to 65% did not change the elution order but

resulted in reduced elution time for all tested compounds, shortening the overall analysis time from 16 min to 6 min (Fig. 2A). Since hydrophobic interactions are partially responsible for column retention of BTEXS, higher methanol contents may have created a more hydrophobic mobile phase environment, favoring the dissolution of BTEXS. This reduces the hydrophobic interactions between the compounds and column stationary phase and hastened elution. The effect was most prominent for compounds with greater hydrophobic character such as toluene, styrene, ethylbenzene and xylene isomers which has octanol–water partition coefficient values (logPOW) between 2.65 and 3.20.[4, 22] The reduction in elution time for the aforementioned compounds was between 6 and 11 min (Fig. 2A). In contrast, the least hydrophobic benzene (logPOW = 2.13) registered a modest 3 min reduction in elution time.

The quality of the separation was evaluated in terms of resolution between adjacent peaks. Fig. 2B shows that 55 to 60% methanol provided high resolution of close to or greater than 1.5 for all tested compounds. Deterioration in resolutions to 1.1 and 1.0 was observed for ethylbenzene/*o*-xylene at 50% methanol and for *o*-xylene/*m*-, *p*-xylene at 65% methanol respectively, producing overlap between the two peaks. As such, 55 to 60% methanol was regarded as most optimal for BTEXS separation. 60% methanol was chosen as the mobile phase for subsequent experiments. An interesting phenomenon was also observed in which the resolution for ethylbenzene/*o*-xylene increased from 1.1 to 1.8 despite a smaller elution time difference of 0.3 min at 65% methanol compared to 0.4 min at 50% methanol (Fig. 2). This outlier is most likely attributed to the changes in π-π selectivity of the stationary phase aromatic group in response to the dielectric constant of the mobile phase [23-24] although further studies will be required to verify this.

Following the optimization of mobile phase, we proceeded to determine the optimal range of injection volume by varying this parameter between 1 and 20 µL. High resolutions (>1.5) between the first four peaks benzene/toluene, toluene/styrene and styrene/ethylbenzene) were observed for all tested injection volumes (Fig. S1). For the last three peaks (ethylbenzene/*o*-xylene and *o*-xylene/*m*-, *p*-xylene) however, chromatographic resolutions close to or above 1.5 were only observed for injection volumes of up to 10 µL. As such, injection volumes between 1 to 10 µL are recommended for concentrated BTEXS samples. High sample injection volumes of 50 µL to 100 µL were frequently reported[12, 14-15] for BTEX separation on C8/C18 columns. However, we found such high sample injection volumes to be detrimental to the Acclaim Phenyl-1 column's lifespan. Column overloading and appearance of residual peaks were observed at injection volumes greater than 50 µL (data not shown). Although attempts to remove residual peaks by flushing the column with 100% methanol were successful, we were unable to restore the column to its former resolving ability. Hence, we advise against overloading the column.

Flow rate is also a parameter known to influence analysis time and chromatographic resolution. Using 60% methanol as the mobile phase, we varied the mobile phase flow rate between 0.8 to 2.0 mL/min to determine the optimal flow rate for rapid analysis without compromising resolution. Increasing the flow rate from 0.8 to 2.0 mL/min, which is the manufacturer's recommended maximum flow rate, halved the analysis time from 12 to 5.5 min (Fig. 3A) with negligible decrease in resolution (Fig. 3B). At a flow rate of 2.0 mL/min, the elution times of benzene, toluene, styrene, ethylbenzene, *o*-xylene, and *m*-, *p*-xylene were 2.5, 3.4, 4.2, 4.6, 4.8, and 5.0 min respectively (Fig. 1B). Compared to existing HPLC methods which

usually have an analysis time of 10 to 15 min[12-15], the proposed method provided time-savings between 5 and 10 min per sample run, leading to higher analysis throughput. This makes the proposed method one of the fastest analytical methods reported to date and is advantageous under circumstances where analysis of large number of samples are required. Furthermore, the amount of solvent required per analysis was about 25% lesser compared to existing methods, generating cost-savings from both solvent usage and disposal.

Analytical parameters including elution time, repeatability, reproducibility and LOD/LOQ, slope, R^2 coefficient, and linearity were also studied under the optimal HPLC operating conditions determined in this work. The HPLC operating conditions were set at a mobile phase of 60% methanol; sample injection volume of 10 μL; and flow rate of 1.2 mL/min. Under these conditions, the elution times of benzene, toluene, styrene, ethylbenzene, *o*-xylene, and *m*-, *p*-xylene were 4.1, 5.7, 6.9, 7.6, 8.0, and 8.3 min respectively (Fig. 1A and Table 1). The operating pressure varied between 170 and 180 bars (data not shown). Repeatability and reproducibility of the method were tested by performing injections of three independent samples on one day and of five independent samples on three days respectively. To evaluate repeatability and reproducibility, the relative standard deviation (RSD) of sensitivity was used. Sensitivity for two tested concentrations (0.02 mM and 2.0 mM) was found to be within RSD values of ±13.6% (Table 1) complying with US EPA quality control criteria. The LOD and LOQ values were expressed as the concentration of analyte that gives a detector signal which is 3 times and 10 times the noise level respectively. Based on this expression, this method was found to be suitable for the detection of trace amounts of toluene, ethylbenzene, styrene and xylenes, at the lower detection limits prescribed by US EPA.[25] The only exception is benzene where the LOD/LOQ

values were above US EPA-prescribed limits and GC-based methods would be required. Within the optimal operating parameters, the LOQ value was expressed as the lower limit of linearity while the upper limit of detection was restricted to 2.0 mM to ensure high resolution, particularly between ethylbenzene and xylenes. High linearity for BTEXS was observed between the lower and upper detection limits with R^2 coefficients of 0.9992 ± 0.0538 or more. Within the confines of the detection limits, the proposed method is most suited for the analysis of highly-polluted effluents.

We tested the application of the proposed method for BTEXS analysis in different aqueous matrices of varying matrix complexity. This ranged from the less complex ultrapure water and tap water matrices to the more complicated matrices including domestic wastewater, and industrial wastewater obtained from a local petrochemical wastewater treatment facility. As non-ionic surfactants such as Pluronic F-68 are commonly used in bioremediation studies to enhance the aqueous availability of volatile monoaromatic compounds[26-27], 2.5% (w/v) Pluronic F-68 surfactant solution was included as one of the test matrix. Test matrices were spiked with BTEXS at two equimolar concentration levels 0.02 mM and 2.0 mM. The influence of matrix in BTEXS analysis was evaluated in terms of mean recovery for three replicates. Recovery values were found to be between 86.4 ± 3.4 and 115.9% ± 5.0 (Table 2) which are within the quality control criteria prescribed for US EPA methods where the acceptable limits for recovery are between 80 and 120%.

We applied the proposed method to analyze a real wastewater sample contaminated by petroleum aromatics products. The wastewater was generated during the cleaning of a plastic

pyrolysis facility. Fig. 4 shows the chromatogram of the wastewater sample which was found to contain benzene, toluene, styrene and ethylbenzene. Specificity of the analysis was evaluated based on match factors and RSD values of peak purity index obtained from Chromeleon v6.80 software (Thermo Scientific Dionex, USA) after comparison against analytical standards. All identified peaks had match factor values between 948 and 989, and peak purity indices with RSD values between 0.02 and 0.31%. GC-FID analysis was performed on the same sample and the results corroborated with that obtained by the proposed HPLC method (Fig. S2). Taken together, these results indicate the high specificity and applicability of the proposed method to actual wastewater samples.

Conclusions

A simple, rapid and reliable HPLC-DAD detection method for the direct analysis of aqueous samples containing high BTEXS concentrations was developed. The optimal HPLC operation parameters were determined to be 55 to 60% methanol for mobile phase, a maximum of 10 µL for sample injection volume, up to 2.0 mL/min for flow rate, and a detection wavelength of 201 nm. Under the optimal conditions, chromatographic separation of concentrated BTEXS samples can be completed within 5.5 min with high resolution, particularly for ethylbenzene and xylenes. To our knowledge, the method is also the first to demonstrate the feasibility of chromatographic separation of styrene together with BTEX, expanding the range of aromatic compounds detected in a single run. Taken together, this method is well-suited for direct aqueous sample analysis without the need for dilution, and can be employed as an alternative method or complement existing GC/HPLC methods for the routine monitoring of highly-polluted BTEXS industrial effluents. There are several advantages of the proposed

method including protocol simplification, high analysis throughput, minimal sample loss, less solvent waste generation and lower price of analysis. It is anticipated that this method will benefit and facilitate the work of field engineers dealing with highly-polluted industrial waste effluents. Additionally, the column and the reported method are compatible with surfactant Pluronic F-68. Pluronic F-68 has wide pharmaceutical applications, particularly in the stability and bioavailability enhancement of drugs; other industrial applications include detergents and personal care product formulations.[28] Hence, beyond the scope of this work, proposed method has immense potential to be adapted and further developed for specific analytical applications involving Pluronic F-68 and its pluronic counterparts.

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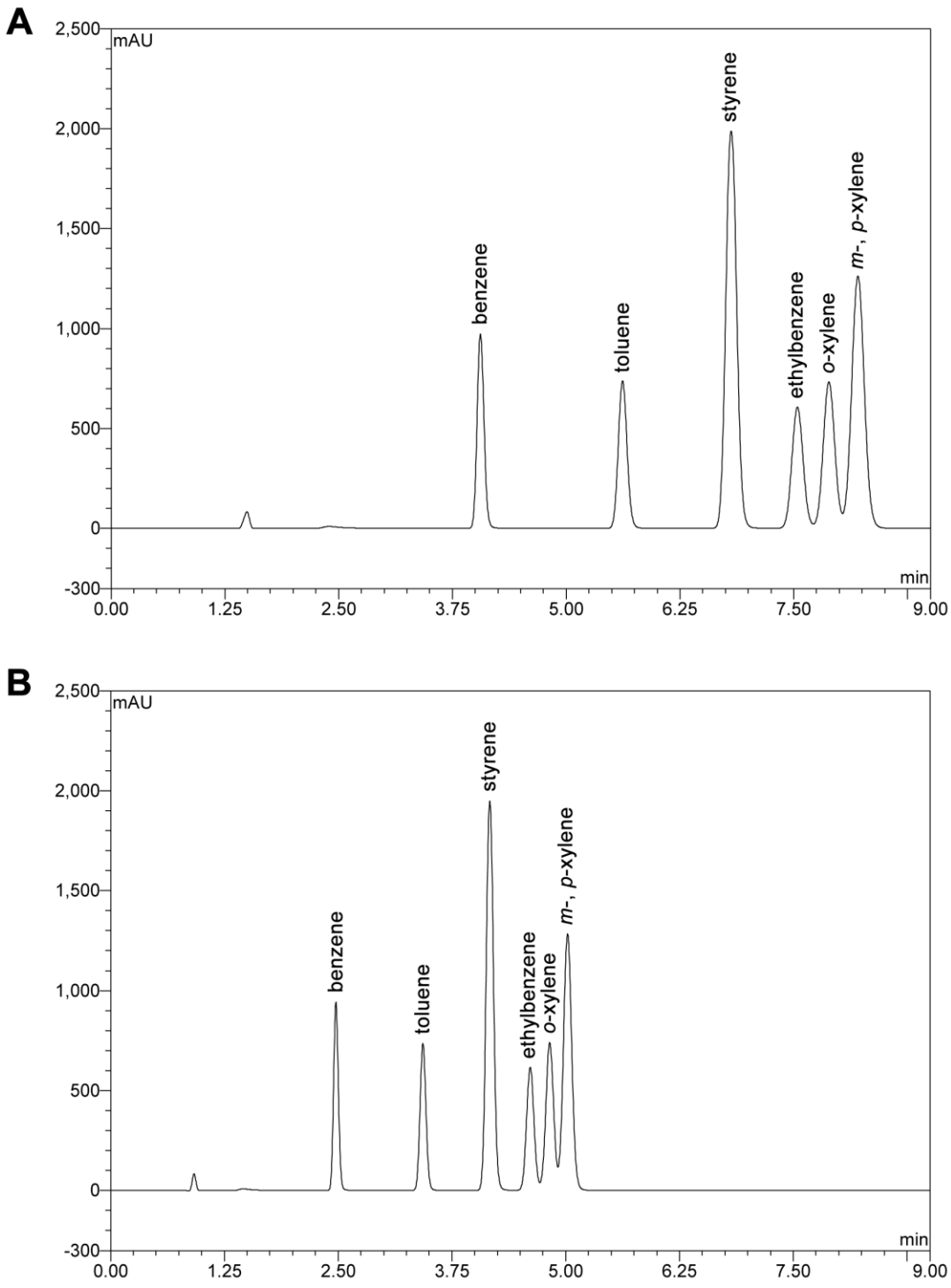

Fig. 1. Chromatogram of a standard 2.0 mM equimolar mixture of BTEXS. Separation was conducted with 60% methanol mobile phase and 10 µL sample injection volume at a flow rate of (A) 1.2 mL/min and (B) 2.0 mL/min.

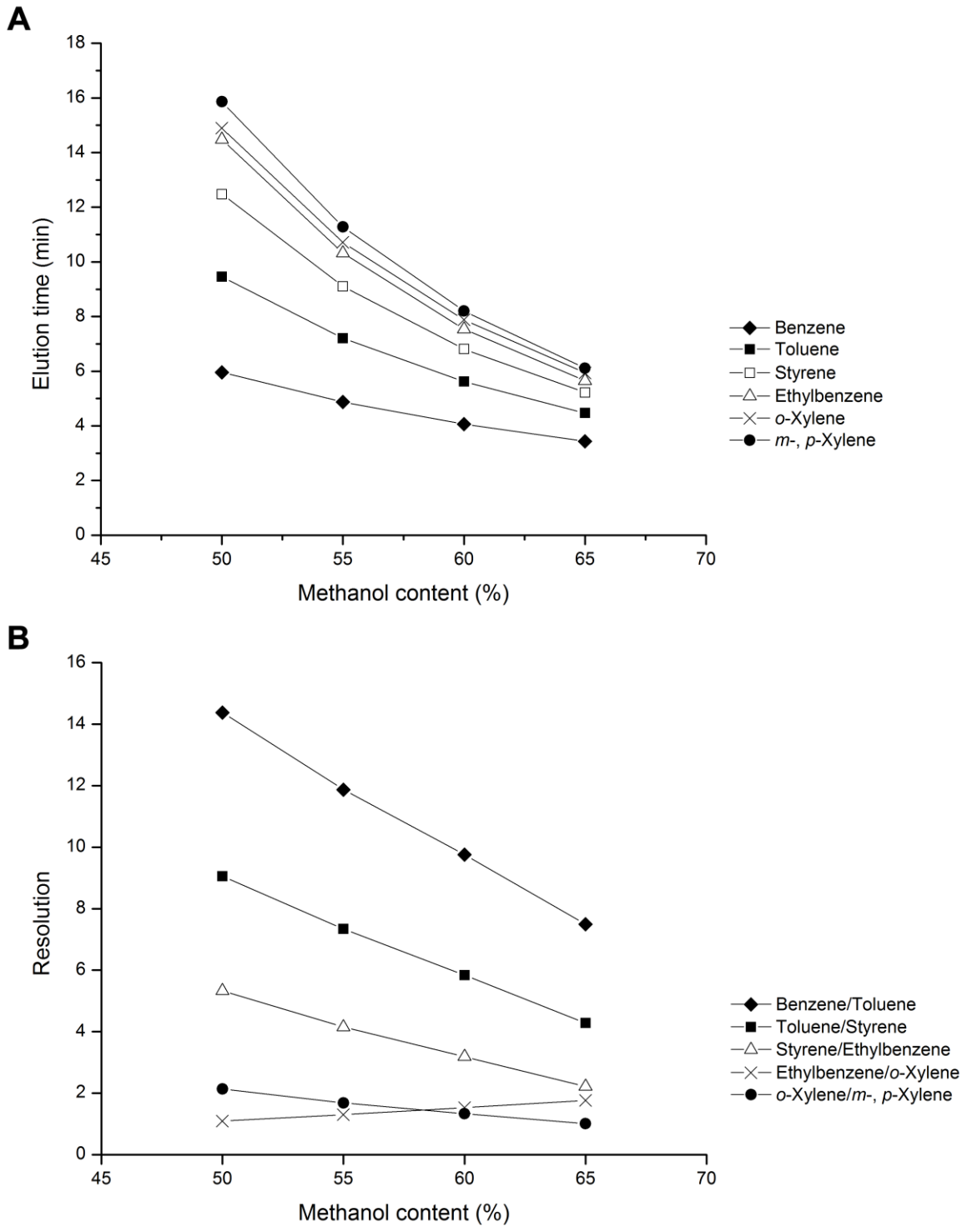

Fig. 2. Effect of methanol content on the (A) elution time and (B) resolution for a standard 2.0 mM equimolar mixture of BTEXS. Sample injection volume and flow rate were 10 µL and 1.2 mL/min respectively. Results are the average of three independent experiments with standard deviations within ±0.006 and ±0.10 for elution time and resolution respectively.

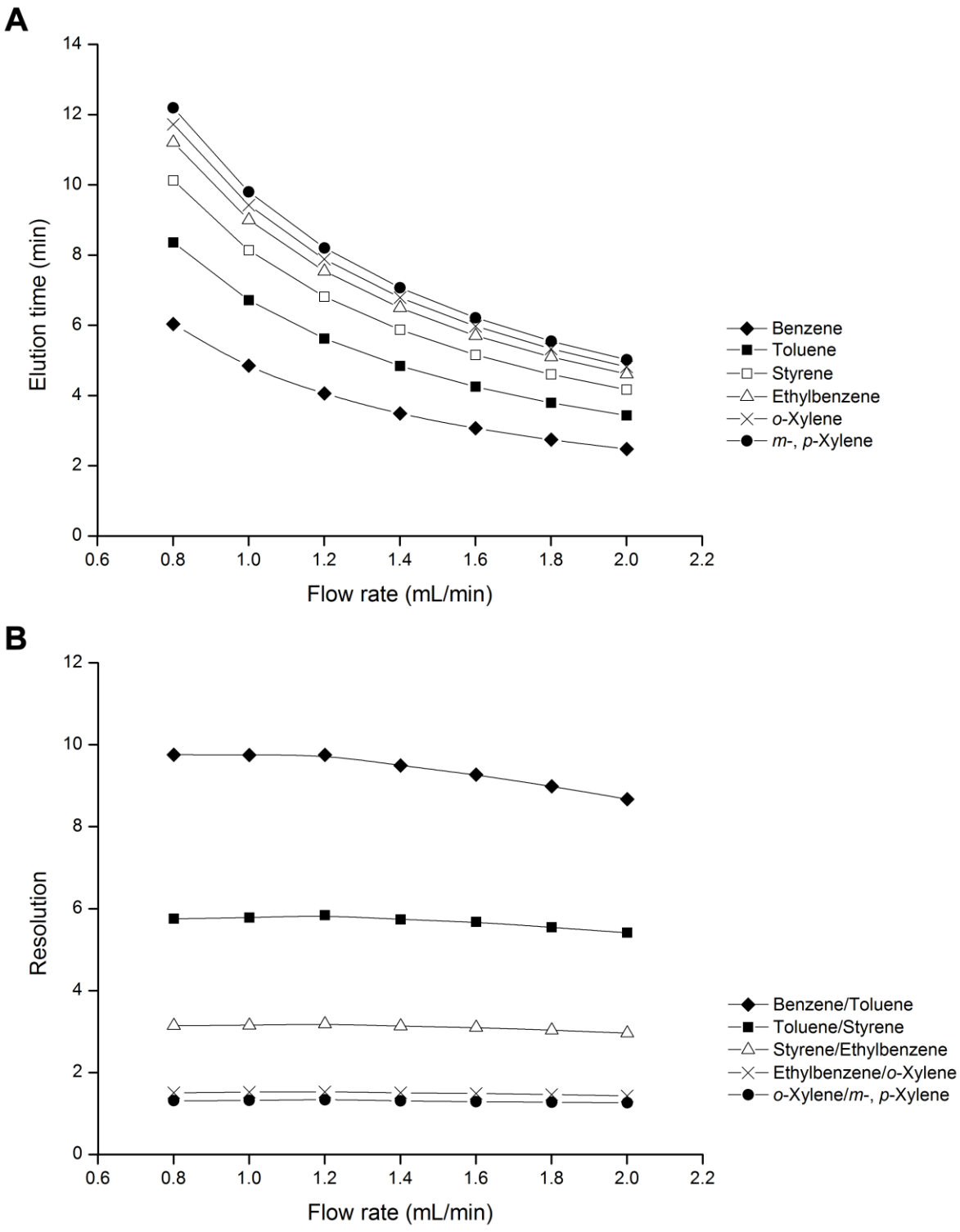

Fig. 3. Effect of flow rate on the (A) elution time and (B) resolution of a standard 2.0 mM equimolar mixture of BTEXS. Separation was conducted with 60% methanol mobile phase and a sample injection volume of 10 µL. Results are the average of three independent experiments with standard deviations within ±0.006 and ±0.09 for elution time and resolution respectively.

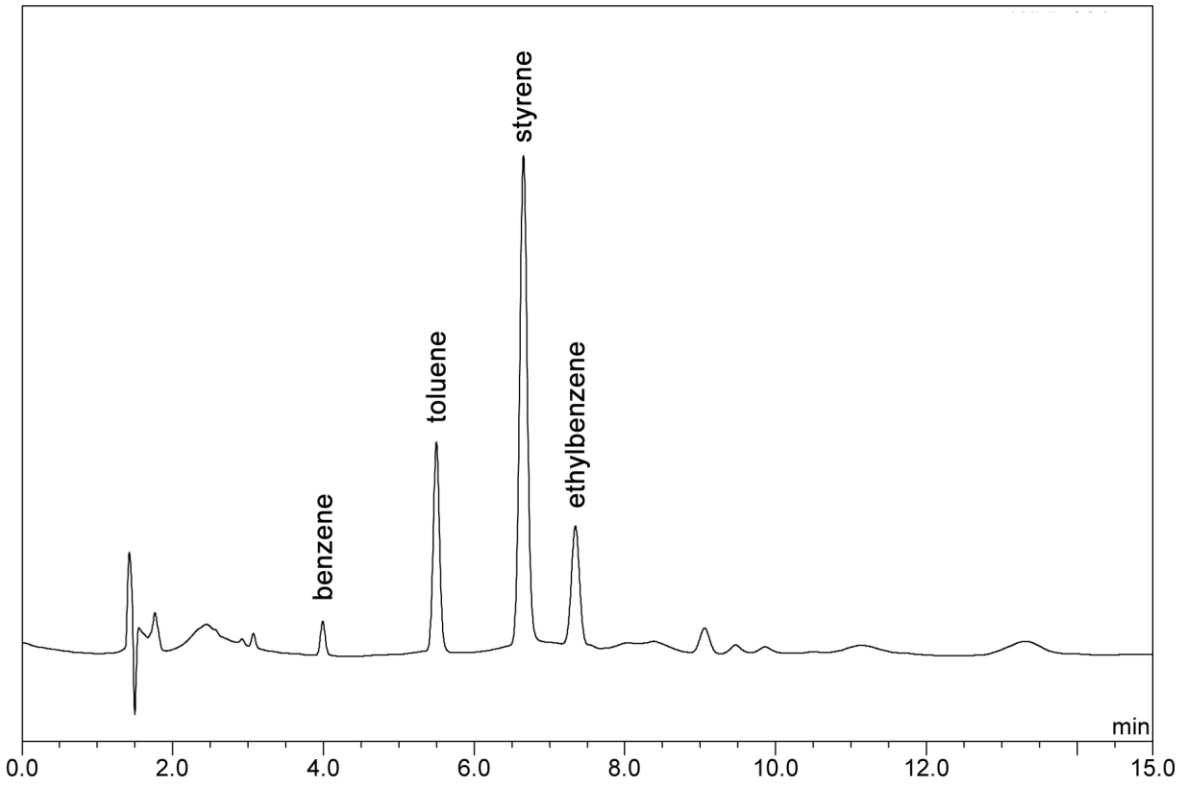

Fig. 4. Chromatogram of a real wastewater sample contaminated by petroleum aromatic products from a plastic pyrolysis facility.

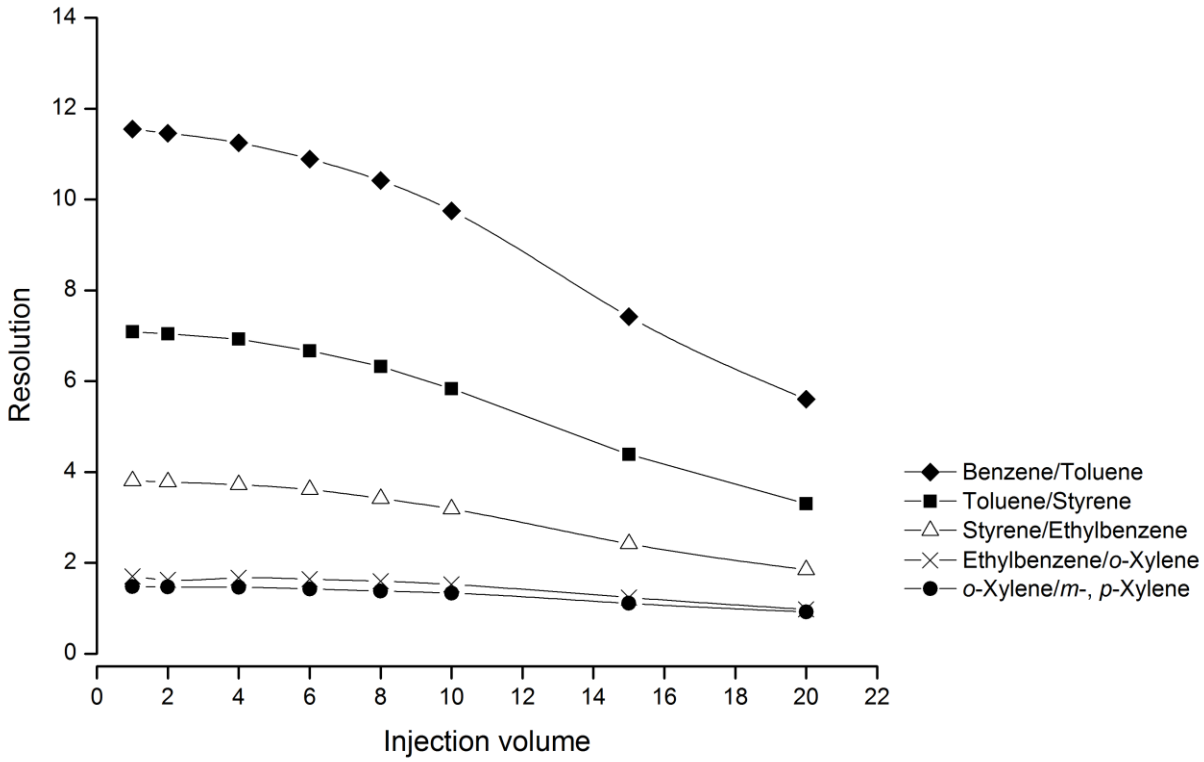

Fig. S1. Effect of injection volume on the resolution of a standard 2.0 mM equimolar mixture of BTEXS. Separation was conducted with 60% methanol mobile phase at a flow rate of 1.2 mL/min. Results are the average of three independent experiments with standard deviations within ±0.12.

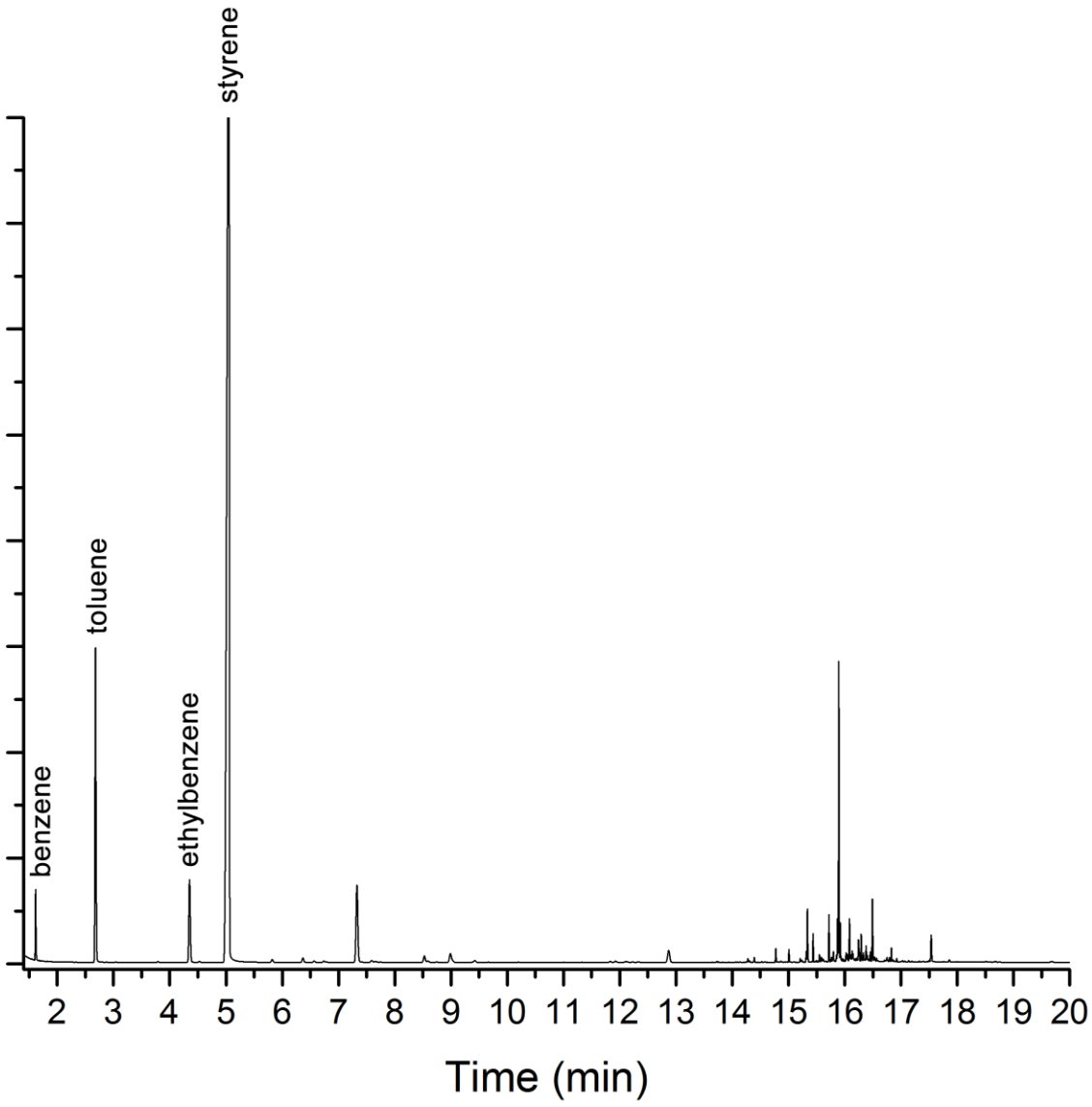

Fig. S2. GC-FID analysis of a real wastewater sample contaminated by petroleum aromatic products from a plastic pyrolysis facility. The elution times of benzene, toluene, ethylbenzene and styrene were 1.6, 2.6, 4.3 and 5.0 min respectively.

Table 1
Analytical parameters of the developed method for BTEXS determination with alkyl aromatic stationary phase separation column

Compound	Elution time (min)	Linear range (mM)	Slope	R^2 coefficient ± RSD[a]	LOD (mM)	LOQ (mM)	Concentration (mM)	Repeatability[b] (RSD%)	Reproducibility[c] (RSD%)
Benzene	4.1	0.001 to 2	63.09	0.9999 ± 0.0143	0.0003	0.001	0.02	7.27	6.30
							2	0.75	1.61
Toluene	5.7	0.001 to 2	67.56	0.9999 ± 0.0071	0.0004	0.001	0.02	6.76	5.10
							2	1.94	2.56
Styrene	6.9	0.0006 to 2	196.40	0.9992 ± 0.0538	0.0002	0.0006	0.02	6.47	4.63
							2	10.60	8.51
Ethylbenzene	7.6	0.002 to 2	65.75	0.9999 ± 0.0060	0.0005	0.002	0.02	6.34	4.62
							2	0.56	2.36
o-Xylene	8.0	0.001 to 2	65.02	0.9999 ± 0.0091	0.0004	0.001	0.02	6.30	13.62
							2	11.27	8.87
m-, *p*-Xylene	8.3	0.0008 to 2	146.58	0.9997 ± 0.0280	0.0002	0.0008	0.02	6.47	4.69
							2	5.62	4.63

[a] Tabulated based on results from five independent samples determination
[b] Tabulated based on response factors from injections of three independent samples performed on 1 day
[c] Tabulated based on response factors from injections of five independent samples performed on 3 days

Table 2
Method recovery in different types of aqueous matrices (n = 3)

Compound	Concentration (mM)	Recovery (%) ± s.d [a]				
		Ultrapure water	Tap water	Industrial wastewater	Domestic wastewater	Pluronic F-68
Benzene	0.02	111.9 ± 6.2	111.3 ± 3.1	112.4 ± 8.2	114.3 ± 7.9	109.8 ± 2.0
	2	94.3 ± 5.1	95.3 ± 4.5	95.1 ± 3.3	96.3 ± 2.3	98.1 ± 2.6
Toluene	0.02	109.4 ± 8.5	109.3 ± 4.4	109.7 ± 10.5	108.7 ± 9.2	106.3 ± 5.4
	2	93.7 ± 5.1	94.0 ± 4.6	94.3 ± 3.7	95.1 ± 3.2	95.3 ± 3.2
Styrene	0.02	112.2 ± 5.5	113.0 ± 6.2	112.5 ± 2.7	115.0 ± 3.2	115.9 ± 5.0
	2	86.7 ± 4.6	86.8 ± 4.0	86.4 ± 3.4	87.4 ± 3.0	87.4 ± 2.1
Ethylbenzene	0.02	111.3 ± 7.6	110.2 ± 7.6	111.3 ± 7.3	112.3 ± 8.9	105.7 ± 10.2
	2	98.6 ± 4.0	98.7 ± 3.6	99.5 ± 2.7	99.9 ± 2.6	99.6 ± 3.2
o-Xylene	0.02	115.9 ± 2.9	109.2 ± 10.3	111.9 ± 7.0	111.4 ± 7.7	113.3 ± 3.4
	2	110.6 ± 3.9	110.5 ± 3.6	112.1 ± 2.9	111.6 ± 2.4	111.1 ± 2.7
m-, *p*-Xylene	0.02	113.5 ± 8.9	109.6 ± 10.1	110.2 ± 8.3	111.2 ± 9.4	112.8 ± 3.6
	2	92.1 ± 4.5	92.2 ± 4.2	92.7 ± 3.5	93.1 ± 2.6	93.0 ± 2.4

[a] s.d. refers to standard deviation