Screening of Liquid Crystal Monomers (LCMs) and Bis(4-chlorophenyl) Sulfone (BCPS) in Human Blood.

LCM and BCPS Method Quality Assurance.

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Sammanfattning

In previous work we performed a retrospective screening for liquid crystal monomers (LCMs) and bis(4-chlorophenyl) sulfone (BCPS) in Swedish adult plasma using an existing high-resolution mass spectrometry (HRMS) dataset. As previously reported to KEMI, no LCMs were detected in that screening exercise, and therefore it was of follow-up interest to confirm that the former analytical method (Xie et al. 2024) was sensitive for this family of analytes. Unlike LCMs, BCPS was tentatively detected in the same retrospective screening, however, it's quantitative recovery by the analytical method was unknown, and we were also concerned that BCPS contamination may come from laboratory consumables used for blood collection or aliquoting. The current work demonstrated that the gas chromatography-HRMS instrumental method used in the former retrospective screening was exquisitely sensitive for all LCMs and BCPS, with a majority of these analytes having instrumental detection limits below 250 femtograms on-column. The overall method detection limits were also very good for BCPS (0.013 ng/mL plasma) and most LCMs (<0.1 ng/mL plasma), which are comparable to values previously reported for priority contaminants with the same method. Unfortunately, recovery of BCPS was low (18%) and outside the quantitative range, thus further method development will be necessary to quantify this substance in human blood. Nevertheless, simulated blood serum samples, collected by passing clean water through representative blood collection and serum aliquoting materials revealed no contamination by BCPS, thereby demonstrating high feasibility for future biomonitoring of BCPS with cooperation of ongoing blood sample collection programmes or national cohort studies.

Background and Purpose.

Liquid Crystal Monomers (LCMs) were identified as a potential group of emerging contaminants by the Toxicological Council in 2023, thus the Swedish Chemicals Agency (KEMI) previously commissioned ACES to undertake a retrospective screening for LCMs in human blood plasma (Project H23-09296). Briefly, in that preliminary project we were unable to detect the presence of 34 prioritized LCMs in any individual or pooled plasma sample. As that project was a retrospective screening in a gas-chromatography high-resolution mass spectrometry (GC-HRMS) dataset, the analyte recoveries and instrumental/method detection limits for LCMs were unknown at the time. The major objectives of the current work were, therefore, to quantify these methodological quality assurance parameters through additional work in the laboratory.

Bis(4-chlorophenyl) sulfone (BCPS) is a high production volume chemical and is manufactured in and/or imported to the European Economic Area. It is used as a monomer in polymers (plastics), such as polysulfones and polyethersulfones, and has been identified as a persistent environmental contaminant that can biomagnify, leading to its inclusion as a *Substance of Very High Concern*.[1] It is a difficult substance to analyze, and to date few human biomonitoring efforts have taken place for BCPS. One attempt to biomonitor BCPS in blood of Europeans (German students) was examined but deemed inconclusive;[2] the MDL was relatively high (2 ng/mL), and an uncontrolled matrix effect was noted that may be due to the presence of co-extracted lipids. Therefore, in the LCM retrospective screening project commissioned by KemI, we also screened for BCPS. Unlike LCMs, BCPS was detectable in some samples above the method blank, however the method blank was not negligible. Due to the widespread presence of BCPS in the environment, and its intentional use in plastics, we hypothesized that it may be present in the blood samples due to contamination during sample collection or storage. Another major objective was therefore to screen for the presence of BCPS and LCMs in simulated blood samples, i.e., clean water drawn through blood sampling equipment and aliquoted into cryotubes.

The project included the following research priorities for BCPS and LCMs:

- Determine absolute percent recoveries of priority LCMs, BCPS, and related internal standards, by the HA-P method[3] (Xie et al. 2024) using spiked human plasma.
- Define an MLOD and MLOQ for LCMs and BCPS by the method of Xie et al., and to the extent possible quantify concentrations in sample extracts previously analysed (KemI contract).
- Convert the nontarget method of Xie et al. (2024) to a more routine target method on GC-MS or GC-MS/MS.
- Analyse for the presence of BCPS in 'blank' samples of blood collection tubing and blood storage tubes that are typically used in biomonitoring and cohorts; if possible using relevant equipment used in the Swedish national HBM-program.

[1] European Chemical Agency. Document: D(2023)3788-DC. May 24, 2023.

[2] Plassmann, M.M., Schmidt, M., Brack, W. et al. Detecting a wide range of environmental contaminants in human blood samples—combining QuEChERS with LC-MS and GC-MS methods. *Anal Bioanal Chem* 407, 7047–7054 (2015). https://doi.org/10.1007/s00216-015-8857-1

[3] Xie et al. 2024. Chemical Exposomics in Human Plasma by Lipid Removal and Large-Volume Injection Gas Chromatography–High-Resolution Mass Spectrometry. *Environ. Sci. Technol.* 58: 17592–17605. https://doi.org/10.1021/acs.est.4c05942; NB, this is the method used to generate GC-HRMS data for retrospective screening in the previous contract research performed for Kemi (Project H23-09296).

Instrumental limits of detection (ILOD).

Rationale and methodology. A mixture of the 34 individual chemicals was prepared in organic solvent. Three concentration levels of the mixture were produced to ensure that all chemicals would render a detectable signal at least once when analysed on the Q Exactive GC-HRMS (Thermo Fisher Scientific). The analysis was done in triplicate with large volume injections (25 µL solvent), as per Xie et al (20245). The analyte peak area corresponding to ILOD was calculated by multiplying the standard deviation of the average analyte signal areas $S_{\bar{x}}$ with the Student's t-table value, t_a = 2.92 (df=2, alpha=0.05), divided by the response factor of the analyte[4].

Results. All chemicals evaluated yielded an acceptable ILOD below 1 pg (1000 fg) on-column (**Figure 1**). Most LCMs were in-fact detectable at levels below 0.25 pg (250 fg), and BCPS had a detection limit of 0.082pg (82 fg) on column. The instrumental method is therefore very sensitive for most analytes.

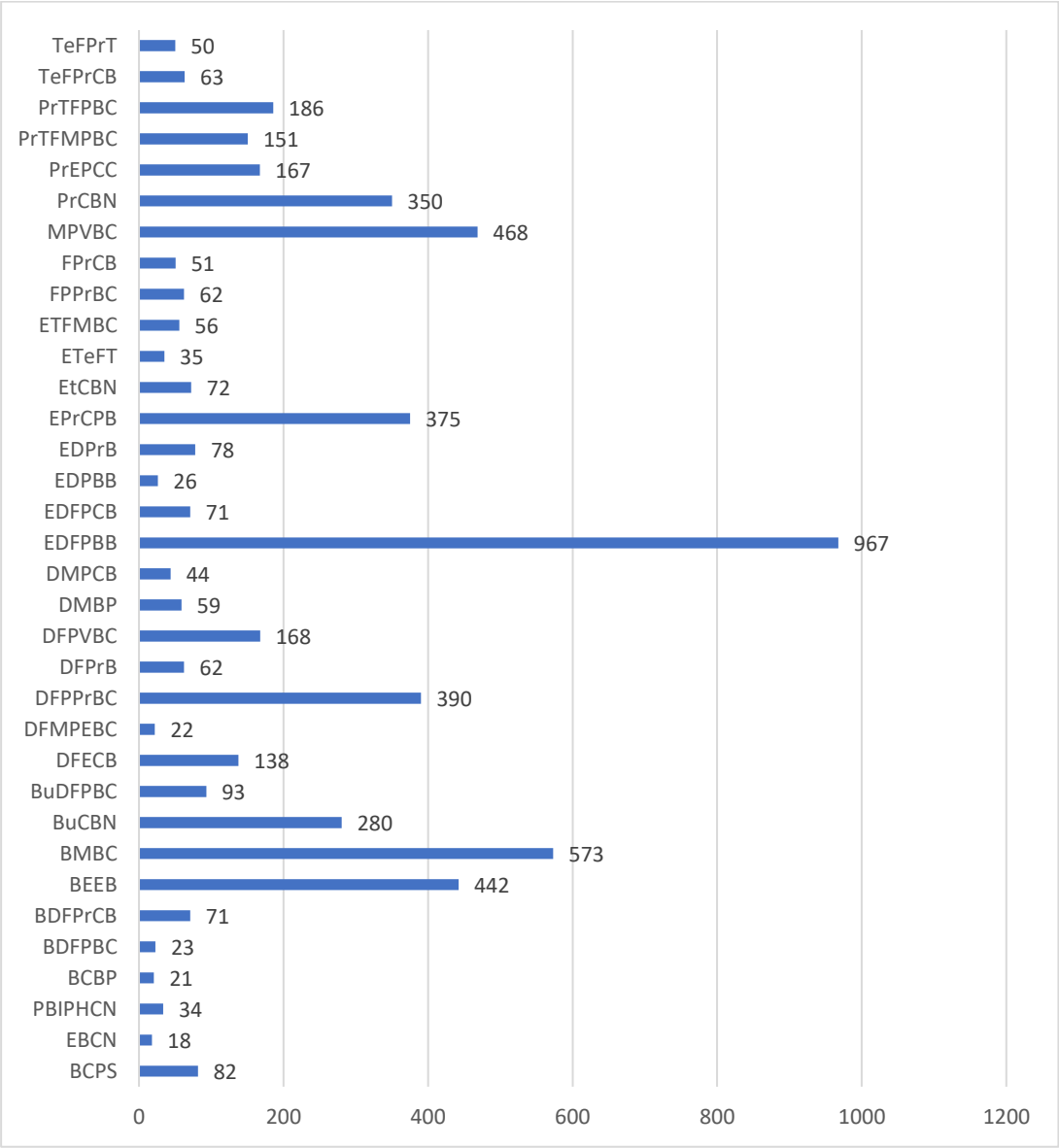


Figure 1. Instrumental limits of detection (ILOD) expressed as femtogram (fg) injected on column.

[4] Agilent Technologies, Inc. "Chemical Analysis Group. Signal, Noise, and Detection Limits in Mass Spectrometry, Technical Note." 2011.

Absolute recoveries and method detection limits (MDLs)

Rationale and methodology. Following the promising ILOD results, which demonstrated a very sensitive instrumental method for BCPS by GC-HRMS, the next step was to determine the absolute recoveries for LCMs and BCPS in the HA-P extraction method of Xie et al. (2024). Four fortified and four non-fortified serum samples (from commercial human serum) were prepared at each of three spiking levels, thus, for each spiking level eight serum samples (200 µL each) were extracted and worked up for GC-HRMS analysis. The three spiking levels were 'Low': 20 ng/L serum (0.02 ng/mL), 'Medium': 1 µg/L serum (1 ng/mL) and 'High': 10 µg/L serum (10 ng/mL). Deuterated methoxychlor (D14 Methoxychlor) was added before GC-HRMS analysis for volumetric normalization. Four additional serum samples and four pure water samples were extracted as solvent blanks checking for background.

Results. BCPS and dimethoxybiphenyl (DMBP; an LCM) were the only two chemicals clearly detectable in the 'Low' fortified and non-fortified serum samples, and also in the non-spiked serum. However, DMBP was also detected in the blank samples (water), likely indicating method contamination. Importantly, BCPS was not detected in the blank samples.

The 'Low' spiking level was set to a concentration that should theoretically, based on IDLs, result in measurable peaks for at least half of the test substances. However, this level is still very low (0.02 ng/mL) and subtle matrix effects and extraction losses may be responsible for the non-detects. As we previously described in Xie et al. (2024), for GC-Orbitrap HRMS spectrometry, the background signal from the blood plasma matrix reduces the ion-filling time of the Orbitrap, which reduces method detection limits in comparison to IDLs determined in solvent.

All LCMs and BCPS were detected at the 'Medium' spike level in serum (**Figure 2**), with most recoveries ranging from 55-81% (n=31 substances). Three chemicals: BCPS, EBCN and PBIPHCN, had poor method recoveries of 18%, 21% and 30%, respectively. The corresponding recoveries for the 'High' spike level were similar (**Figure 2**), with most recoveries ranging from 46-73% (n=31 test substances), while BCPS, EBCN and PBIPHCN had lower recoveries of 14%, 17% and 26% respectively.

Based on the recovery data for spiked human serum, it can be confidently concluded that the HA-P method with GC-HRMS analysis resulted in method detection limits (MDLs) <1 ng/mL plasma for all LCMs, and an MDL <0.02 ng/mL plasma for BCPS. With extrapolation from the recovery experiments, the true MDL for BCPS was estimated to be 0.013 ng/mL plasma, and for most LCMs were <0.1 ng/mL plasma (exceptions were MPVBC: 0.281 ng/mL, and DMPCB: 0.150 ng/mL). As important context, in the validated chemical exposomics method of Xie et al. (2024), the median method quantification limit was 0.09 ng/mL, thus the current MDLs are very much comparable to what we previously reported for POPs and priority contaminants in human plasma. This provides very important context that previous retrospective screening of LCMs and BCPS in plasma from Swedish adults was with a highly sensitive nontarget analytical method.

The suite of internal standards used here, and by Xie et al. (2024), are well recovered by the method can therefore be used to improve quantification accuracy of most LCMs in future work (exception EBCN and PBIPHCN). For GC-HRMS analysis, we recommend choosing the internal standard with the closest retention time, however, this was not demonstrated here because we have not previously detected any LCMs in samples. For BCPS, which was not well recovered, the current suite of internal standards cannot be used to correct for the poor recoveries. There is no commercial supply of an isotope-labelled BCPS, and our search for Departmental stocks could not identify useful analogues. Future work will instead focus on improved recovery into the acetonitrile fraction and LC-HRMS analysis.

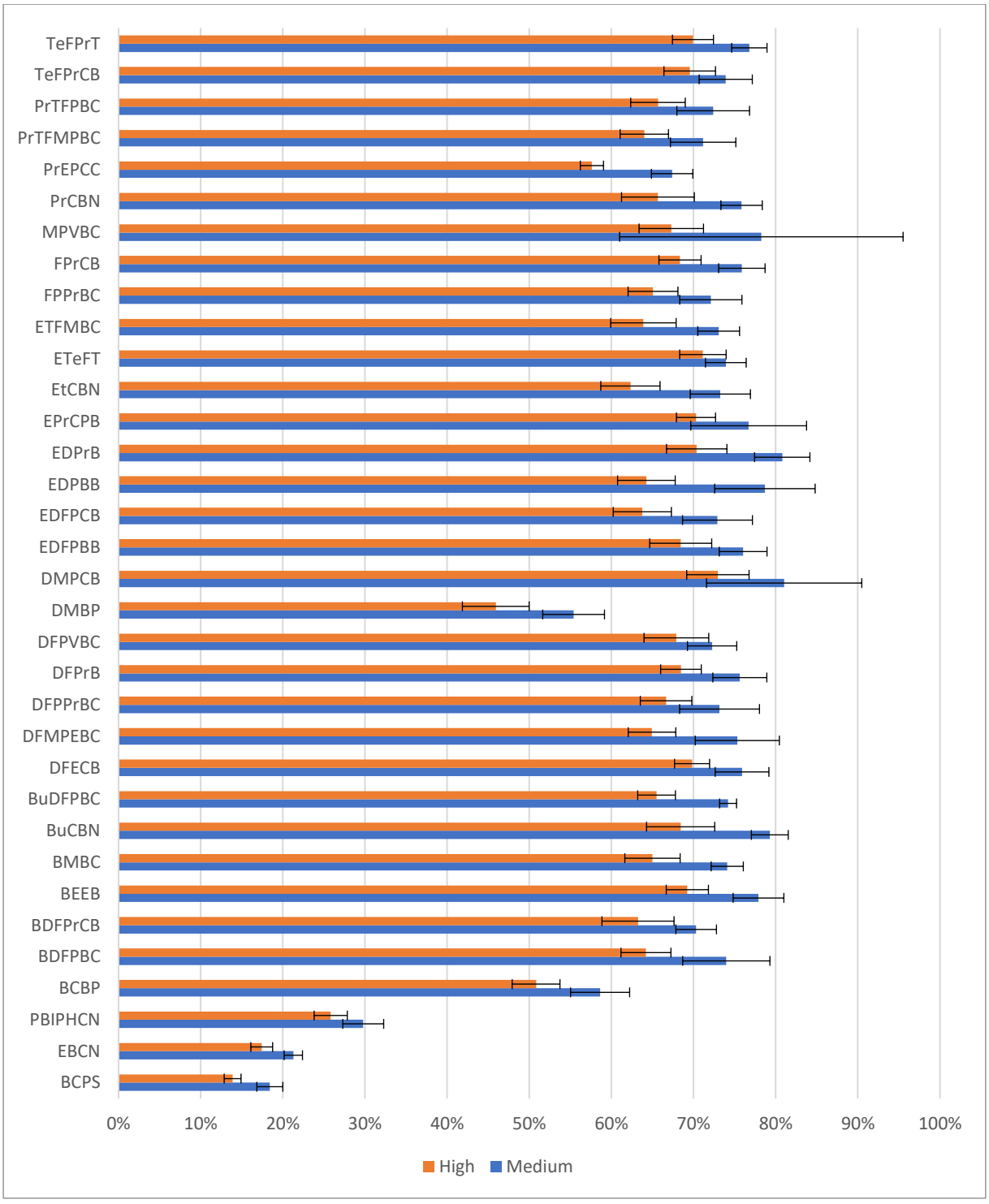


Figure 2. Recovery of LCMs and BCPS from fortified human serum at the medium and high concentration levels (n=4 replicates at each level, showing mean value ± SD). Data are not corrected for recovery standards, but are normalized to the volumetric internal standard peak area.

Testing blood-collection and serum aliquoting equipment for background levels of BCPS.

Rationale. BCPS was detected in authentic human plasma samples, above blank levels, as reported to KemI in 2024. Confidence in the real-world relevance of these retrospective data currently remains tentative because of the known use of BCPS in common plastics, some of which may be used in blood collection or serum aliquoting/storage equipment. Here, by testing relevant blood collection and serum aliquoting materials for background levels of BCPS, we provide the first data on the potential for BCPS contamination during blood sample collection and storage. For this experiment we did not have access to the specific devices and equipment used for collection of the blood where BCPS has been tentatively detected, but for maximum relevance we requested sampling devices and serum aliquoting equipment currently being used in the Swedish POPUP cohort (contact: Sanna Lignell, Biomonitoreringssamordnare, Livsmedelsverket). The collection materials received from Livsmedelsverket are shown in **Table 1**.

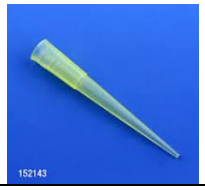
Methodology. Three combinations of three collector tubes and needles were tested in such a way that if a positive contamination was to occur, we may be able to pinpoint the source.

1. Combination 1: Vacuette quick + 8 mL collector tubes.
2. Combination 2: Vacuette safety + 8 mL collector tubes.
3. Combination 3: Vacuette quick + 6 mL collector tubes.

For all other sample handling and extraction, the same materials (i.e. tubes and tips) and water sample were used for each combination. HPLC grade water was drawn through the needles into the different vacuum collector tubes, the vacutainers were inverted once and allowed to sit for 30 minutes, to simulate blood clotting. After centrifugation, approximately 5 mL of water (“serum”) was transferred to Pyrex test tubes and put in a -20 °C freezer overnight. The next day an aliquot of 0.5 mL was transferred to a 2 mL cryotube and the samples stored in a -80 °C freezer until they could be processed for extraction and analysis following the method described by Xie et al. (2024). Briefly, in a test tube, internal standards (if any), are added to a 200µL serum sample followed by protein precipitation with 800 µL acetonitrile. The sample is vortexed for a minute followed by centrifugation at 2500g for 5 minutes. The supernatant is transferred to a new test tube and is in turn extracted with two portions of isohexane (1200 µL + 600 µL). The combined hexane is collected in an empty test tube, and after careful solvent evaporation to approximately 100 µL, a normalizing volumetric standard is added and the finished sample is transferred to an analysis vial with 300 µL insert. Automated large volume injections of 25 µL are done on a Q Exactive GC system equipped with a PTV injector and a 30 m DB-5 MS ultra-inert column (Agilent.com #122-5522UI). Thermo scientific software, Xcalibur, was used to control the instrument and also used for collecting and analysing all data.

Results: No traces of BCPS were detected in simulated serum aliquots collected through the above noted devices and stored in cryovials.

Table 1. Description and images of blood sampling equipment used for testing the blank levels of BCPS in simulated blood sampling and serum aliquoting.

Equipment Description	Image / Photo
Vacuette Quickshield complete PLUS 21G x 1 ½, safety tube holder.	
Vacuette SAFETY Blood collection set + holder 23G.	
BD Vacutainer clot activator tube, 10 mL.	
Vacuette serum clot activator tube, 6 mL.	
Pyrex test tubes for storage of samples in -20 °C.	
Thermo Fisher pipette tips, 200 µL with filters used for dispensing serum to cryotubes.	
Cryo-tubes 2.0 mL size for storing serum samples in -80 °C.	

Summary and Conclusions

In previous work we performed a retrospective screening for liquid crystal monomers (LCMs) and bis(4-chlorophenyl) sulfone (BCPS) in Swedish adult plasma using an existing high-resolution mass spectrometry (HRMS) dataset. As previously reported to KEMI, no LCMs were detected in that screening exercise, and therefore it was of follow-up interest to confirm that the former analytical method (Xie et al. 2024) was sensitive for this family of analytes. Unlike LCMs, BCPS was tentatively detected in the same retrospective screening, however, it's quantitative recovery by the analytical method was unknown, and we were also concerned that BCPS contamination may come from laboratory consumables used for blood collection or aliquoting. The current work demonstrated that the gas chromatography-HRMS instrumental method used in the former retrospective screening was exquisitely sensitive for all LCMs and BCPS, with a majority of these analytes having instrumental detection limits below 250 femtograms on-column. The overall method detection limits were also very good for BCPS (0.013 ng/mL plasma) and most LCMs (<0.1 ng/mL plasma), which are comparable to values previously reported for priority contaminants with the same method. Unfortunately, recovery of BCPS was low (18%) and outside the quantitative range, thus further method development will be necessary to quantify this substance in human blood. Nevertheless, simulated blood serum samples, collected by passing clean water through representative blood collection and serum aliquoting materials revealed no contamination by BCPS, thereby demonstrating high feasibility for future biomonitoring of BCPS with cooperation of ongoing blood sample collection programmes or national cohort studies.