novel two-dimensional gas chromatography approach for prediction of mutagenicity of bitumen and bitumen fumes

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Introduction In 1999 Shell published a fast and fully automated instrumental method developed to produce an analytical marker, correlating chemical characteristics, rather than individual compounds, with mutagenicity and carcinogenicity. The method was based on a Flow-Injection Analysis (FIA) version of the manual cyclohexane-dimethylsulfoxide (DMSO) liquid-liquid extraction method standardized by the Institute of Petroleum as IP-346, which selectively extracts all the potentially carcinogenic Polycyclic Aromatic Compounds –PACs- (i.e. those containing no, or only a few substituents). The use of FIA had a number of advantages in comparison with the manual extraction. It is fast, easy to automate and requires only small amounts (milligrams) of sample. The last feature opened up the ability to perform measurements on fumes released during the hot application of bitumen-containing material as, for instance, in road paving and roofing. Gas chromatography with flame-ionization detection was used to determine the total amount of material extracted. The method was extended towards a general, automated method that allowed the determination of PAC content of oil products, regardless of their boiling range, through incorporation of a Normal-Phase (NP) LC step. The latter served to separate and remove DMSO and any possible co-extracted residual materials showing very little or no biological activity. Results obtained on a wide range of petroleum based materials, have been demonstrated to correlate well with mutagenicity and carcinogenicity Measurements. The main drawback of the approach, however, is the sheer complexity of the experimental set-up. A potentially attractive, and by now, far more simple means to gain access to similar data is now offered through the utilization of comprehensive two-dimensional gas chromatography (GCxGC). In GCxGC, two independent GC separations are applied to the entire sample. The sample is first separated on a high-resolution capillary GC column in the programmed temperature mode. Using a device called a thermal modulator, fractions of effluent from this first column are focused at regular, short intervals and injected onto a second capillary column, which is short and narrow to allow very rapid, isothermal, separations . The resulting chromatogram can be represented as a two-dimensional image from which the peaks emerge. One dimension represents the retention time on the first column, the second one represents the retention time on the second column, and the third one represents the detector's signal intensities in false color. In contrast to the already well established heart-cut two-dimensional GC (GC-GC), in GCxGC the separations in the two dimensions can be made completely independent of each other, or orthogonal. Because of this orthogonality, ordered separations can be expected to occur if the proper separation dimensions are chosen. This means that a very substantial fraction of the theoretical peak capacity can actually be exploited in practical separations . It is this ability to create 'order' in a chromatogram that is one of the strongest assets of GCxGC . Compounds can readily be sorted according to chemical or molecular functionality, structure or shape (i.e. number and geometry of aromatic rings, carbon substitution), which makes identification reliable and relatively easy . Figure 1. Conceptual GCxGC separation of aromatic species; FIA-DMSO equivalent data can be produced by applying weighing-factors on concentrations of substituted PACs to mimic DMSO extraction efficiency A properly chosen combination of columns will give rise to an ordered separation of PACs according to the number of aromatic rings in the second dimension and according to their carbon substitution in the first dimension, as depicted in Figure 1. After integration of the relevant PAC groups, the DMSO extractability, which exhibits a linear relationship with the fraction of aromatic carbon1, can be mimicked by applying weighing factors. Sample introduction using a programmable temperature vaporizer (PTV) enables selective exclusion of "non-volatiles" . In the FIA-DMSO set-up this was to be realized by incorporation of the NPLC step. Obviously, the current use of a PTV to this purpose is a far more simple approach, effectively making it simply part of the sample-introduction procedure. Materials and Methods The GCxGC system used was based on an Agilent 6890 series GC equipped with a Gerstel CIS-4 PTV injector, a CTC-PAL multi-purpose sampler and a flame-ionization detector. To enable the GCxGC experiment a loop-type liquid-nitrogen cryogenic modulator set to a modulation time of 10 seconds and a second dimension column oven were used. The column-set used consisted of a 10 m x 0.25 mm I.D. DB-1 column with a film thickness of 0.10 µm used as first dimension, a 2 m x 0.1 mm I.D. DPTMDS deactivated fused-silica modulation capillary and a 80 cm x 0.10 mm I.D. BPX-50 column with a film thickness of 0.05 µm used as second dimension. The GC oven (first dimension and modulation capillary) was programmed from 100 °C (5 min isothermal) to 350 °C (10 min isothermal) at 2 °C/min. The second-dimension column was programmed from 150 °C (5 min isothermal) to 370 °C (25 min isothermal) at 2 °

C/min. The temperature of the modulator's hot-pulse was programmed from 150 °C (5 min isothermal) to 350 °C (35 min isothermal) at 2 °C/min. 1 µL aliquots of a 10 % oil sample in cyclohexane were injected into the PTV, which was programmed from 40 °C (0 sec isothermal) to 350 °C (10 min isothermal) at 12 °C/sec. After finishing this temperature program, the PTV was cooled down to 40 °C, effectively trapping non-volatiles in its glass-wool-packed glass liner. A head-pressure of 200 kPa (Helium) was applied and a split-flow of 20 mL/min was used. An EZChrom Elite, version 3.1-chromatography data system, acquired the FID signal. MatLab version 6.5 was used for conversion of the linear signal into two-dimensional data matrices. GCxGC data was processed with in-house developed software . The GCxGC data was visualized using Noesys Transform v. 3.0. Results and Discussion In Figure 2 a GCxGC chromatogram of a high-PAC-content deasphalted residual sample is presented. The different classes of PACs are arranged in an orderly fashion according to boiling point in the first dimension, and according to polarity (number of aromatic rings) in the second dimension. Concentrations of PACs are indicated through false-color display of the detector-signal intensity. PAC isomers are grouped in roof-tile like clusters. After integration through in-house developed software10, the concentrations of the different PAC groups can be determined. By applying weighing-factors to mimic the DMSO extractability, it should be possible to generate data similar to those obtained by the current FIA-DMSO set-up. The excellent correlation of the results obtained from the latter with mutagenicity measurements3, gives high promises for the proposed method. A series of samples on which Mutagenicity Indexes have been established and FIA-DMSO data has been generated, is currently under investigation. Figure 2. False-color GCxGC chromatogram of a deasphalted residual oil sample demonstrating ordered class-separation of the different PACs according to number of aromatic-rings and carbon-substitution References J. Blomberg, P.C. de Groot, H.C.A. Brandt, J.J.B. van der Does, P.J. Schoenmakers, J. Chromatogr. A, 849 (1999) 483-494 IP346/92 (98), Polycyclic Aromatics in Lubricating Base Oils and Asphalthene Free Petroleum Fractions – Dimethyl Sulphoxide Extraction Refractive Index Method (ST-G-2), Institute of Petroleum, London (1998) H.C.A. Brandt, P.C. de Groot, J. Blomberg, Polycyclic Aromatic Compounds, 16 (1999) 21 J.B. Phillips and J. Xu, J. Chromatogr. A, 703 (1995) 327 J.C. Giddings, J. Chromatogr. A, 703 (1995) 3 E.B. Ledford Jr., J.B. Phillips, J. Xu, R.B. Gaines and J. Blomberg, Amer. Lab., 6 (1996) 285 J. Blomberg, J. Beens, P.J. Schoenmakers, and R. Tijssen, J. High Resol. Chromatogr., 20 (1997) 539 J. Beens, J. Blomberg, P.J. Schoenmakers, J. High Resol. Chromatogr., 23 (2000) 182 Diane Nicholas, Procedure of using the OPTIC 2 for Selective Exclusion, ATAS Chromatography Technical Note 18 A, ATAS, Cambridge, UK V.G. van Mispelaar, A.C. Tas, A.K. Smilde, P.J. Schoenmakers and A.C. van Asten, J. Chromatogr. A, 1019 (2003) 15-29